# Biological Substrates: The Role of Metal Ions in their Synthesis and Reactivity

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by

Dierdre Anne Pearce

Research School of Chemistry
Canberra A.C.T.

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The work presented in this thesis
is the original work of the candidate
except where otherwise indicated

Dierdre Pearce

Dierdre Pearce

This work is dedicated to Mrs Sue Channon

and all other talented, dedicated, enthusiastic teachers of chemistry.

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# Abstract

This thesis examines reactions of α-imino acids and phosphate esters that are coordinated to Co(III). The metal centre activates certain sites in these species and provides a means of conducting the chemistry under mild conditions.

Sodium dithionite, $Na_2S_2O_4$, was used to reduce a range of α-imino acid complexes, $[N_4Co(NH{=}C(R)COO)]^{2+}$, where $N_4$ indicates tetraamine co-ligand(s). N-alkyl imino acid complexes, $[N_4Co(N(R'){=}C(R)COO)]^{2+}$ were not reduced by dithionite ion. Reduction of the metal centre also occurred but could be minimised by control of the pH of the solution (pH $\leq$ 4.5) and the selection of the co-ligand. Some stereoselectivity in the reduction of $[(en)_2Co(NHC(R)COO)]^{2+}$ complexes was observed. This was greatest in the instance of the imino acid complex $[(en)_2Co(val\text{-}im)]^{2+}$, where the isomer distribution of the product $[(en)_2Co(val)]^{2+}$ was ΛR, ΔS : ΛS, ΔR = 4.2 : 1.0. A reaction mechanism, involving initial addition of $S_2O_4^{2-}$ to the α-carbon of the imino acid, followed by loss of $SO_2$ and hydride transfer from the residual $HSO_2$ moiety, is proposed to account for these findings.

A series of α and β regioselectively deuteriated α-amino acids was prepared by reducing the corresponding α-imino acid with $NaBD_4$ and/or exchanging the protons on the β-carbon for deuterium in $OD^-$ solutions. Labelled species such as these are useful tools in studies of the structure and biosynthesis of secondary metabolites.

The deprotonated β-carbon of $[N_4Co(ala\text{-}im)]^{2+}$ proved to be a good nucleophile for addition to the α-carbon of the imine species, $[N_4Co(HN{=}C(CH_3)COO)]^{2+}$. This reaction generated initially a new binuclear amino acid complex having a polyfunctional side chain. It readily lost a Co(III) centre, however.

The imine-N of $[(en)_2Co(ala\text{-}im)]^{2+}$ was alkylated by *trans*-1,4-dibromo-2-butene. A second addition-elimination reaction with a co-ligand-N resulted, stereospecifically, in a new imino acid complex bearing a pendent alkene which may be further functionalised or polymerised to generate new materials with potentially useful and interesting properties.

The activation of the phosphorus atom of phosphate derivatives upon coordination to a metal centre was used to examine a number of reactions involving attack by coordinated nucleophiles on the phosphorus centre. In some initial investigations, pyrophosphate was synthesised by template addition of coordinated phosphate to coordinated 4-nitrophenylphosphate. The yields of such reactions were not high, but the experiments provide an indication of how polyphosphate derivatives might be synthesised under very mild conditions. The desirability of such syntheses is, of course, evident.

Carbamoyl phosphate is an important intermediate in the biosynthesis and degradation of biological nitrogenous substances such as amino acids and nucleotide bases. $[(NH_3)_5Co(OPO_3CONH_2)]^+$ was hydrolysed by coordinated hydroxide ion using the reagent $[tamenCo(OH)(OH_2)]^{2+}$. $^{18}O$ tracer experiments demonstrated that hydrolysis occurred via addition to the phosphorus, not the carbon centre. This is a facile coordinated process but it does not mimic the biological cleavage. However, hydroxide ion in the $[tamenCo(OH)(OH_2)]^{2+}$ reagent was also found to hydrolyse the phosphodiester backbone of DNA. The rate of hydrolysis was found to be dependent on the type of Co(III) complex, in keeping with previous studies of hydrolysis of simpler phosphodiesters and it also occurred with some regiospecificity.

# Abbreviations

| | |
|---|---|
| A | ammonia |
| ADP | adenosine 5'-diphosphate |
| ala | alanine |
| 3'-AMP | adenosine 3'-monophosphate |
| 5'-AMP | adenosine 5'-monophosphate |
| ATP | adenosine 5'-triphosphate |
| BNPP | bis(4-nitrophenyl)phosphate |
| bipy | 2,2'-bipyridine |
| BIS-TRIS | bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane |
| cAMP | cyclic(-)-adenosine 3', 5'-monophosphate |
| cyclen | 1,4,7,10-tetraazacyclododecane |
| DNA | deoxyribonucleic acid |
| en | ethane diamine |
| glu | glutamic acid |
| gly | glycine |
| HEPES | N-2-hydroxyethylpiperazine-N'-ethane sulfonic acid |
| leu | leucine |
| lys | lysine |
| MES | 2-(N-morpholino)ethane sulfonic acid |
| met | methionine |
| $N_4$ | tetraamine coligand |
| nmr | nuclear magnetic resonance |
| Pi | orthophosphate |
| PPi | pyrophosphate |
| pip | piperidine |

| | |
|---|---|
| $PNP^-$ | 4-nitrophenylate ion |
| pro | proline |
| RNA | ribonucleic acid |
| sar | sarcosine |
| tamen | 6-methyl-6-(4-amino-2-azabutyl)-1,4-diazacycloheptane |
| tn | 1,3-propanediamine |
| NaTPS | sodium-3-(trimethylsilyl)-propanesulphonate |
| tren | N,N-bis(2-aminoethyl)-1,2-ethanediamine |
| trien | N,N'-bis(2-aminoethyl)-1,2-ethanediamine |
| trpn | tris(3-aminopropyl)amine |
| tyr | tyrosine |
| val | valine |

Chromatography: The dimensions of all columns of ion exchange resin are given in the form (a x b cm), where a = diameter and b = length.

# CHAPTER 1

# An Introduction to the Field

## General Outline

One of the interesting developments in chemistry has been the use of metal ions to activate organic molecules and to organise 'template' syntheses between organic fragments around the metal ion. This thesis explores the use of metal ion templates to activate and synthesise biologically relevant molecules. Important considerations include determination of reaction pathways and regio- and stereo- selective control of the products. The reactions described in the following chapters include examples which aim to mimic biological processes and those which synthesise phosphate derivatives and novel amino acids.

This chapter provides background information about the biological roles of α-amino acids and phosphate esters, about the design of complexes and reactions of coordinated ligands which preceded the material presented in the following chapters.

## Biochemistry of α-Amino Acids and Phosphate Esters

### *BIOCHEMISTRY OF α-AMINO ACIDS*

#### *General Structure and Synthesis*

Naturally occurring α-amino acids have the general form **1**. They are synthesised from glycolytic, pentose phosphate or citric acid cycle intermediates or by modification of α-amino acids that were synthesised from these intermediates.[1] For example, alanine arises from a transamination reaction which transfers an amino group from glutamate to pyruvate, **2**. The same type of reaction yields aspartate (**5**) from oxaloacetate, and asparagine (**6**) is synthesised from aspartate and $NH_4^+$.

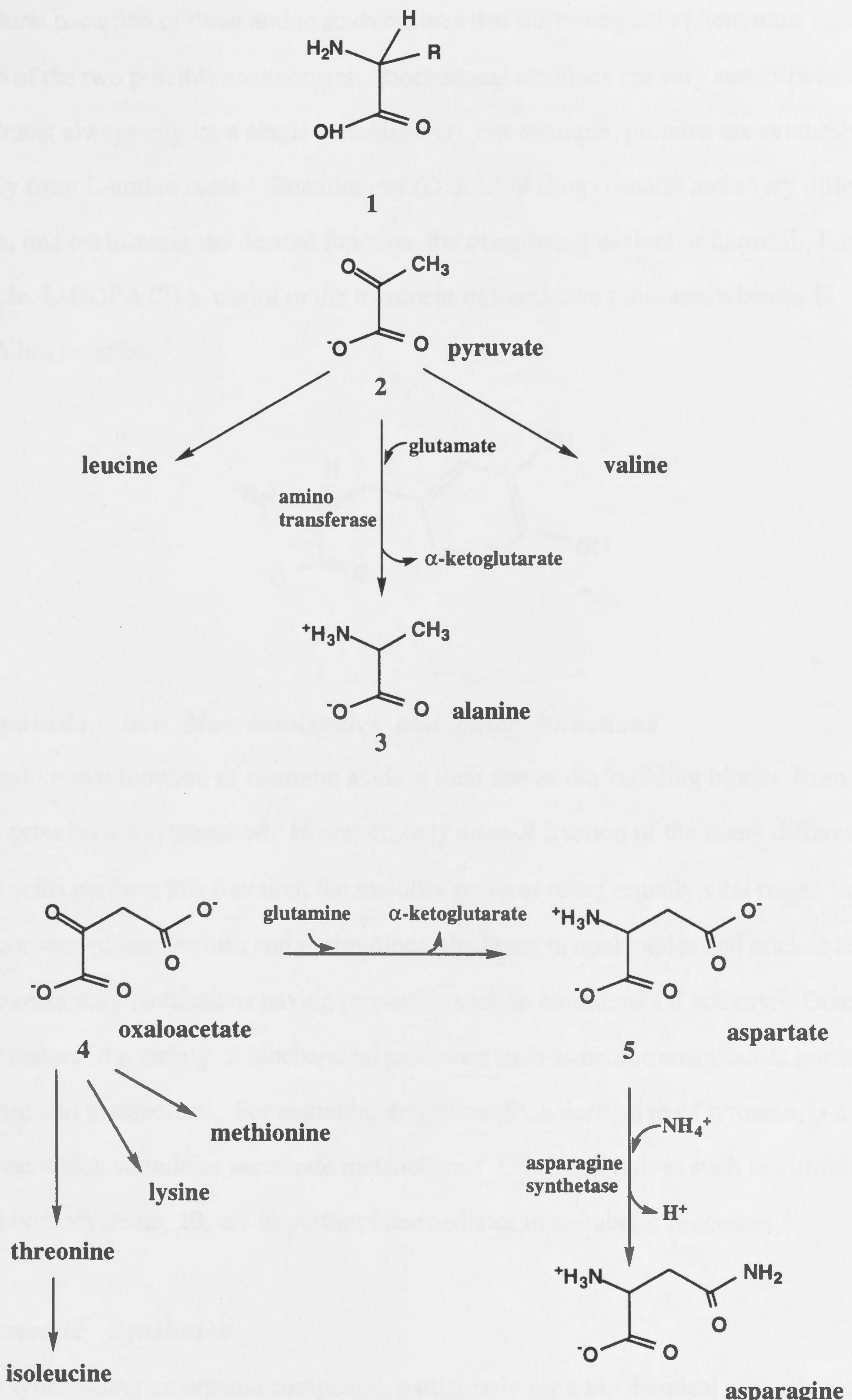
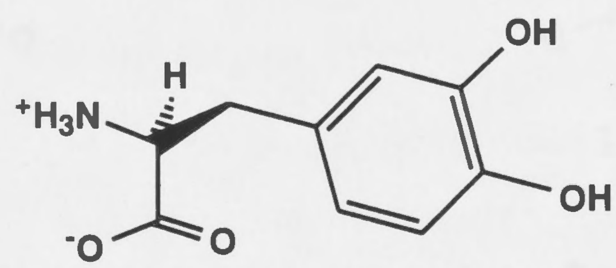



Figure 1: Biosynthesis of some α-amino acids.[1]

The chiral α-carbon of these amino acids ensures that the biological system must make a choice of the two possible enantiomers. Biochemical reactions are very stereospecific and almost always require a single stereoisomer. For example, proteins are synthesised entirely from L-amino acids.[1] Enantiomers (D or L) of drugs usually have very different effects, one performing the desired function, the other being useless or harmful. For example, L-DOPA (**7**) is useful in the treatment of Parkinson's disease, whereas D-DOPA has no effect.[1]

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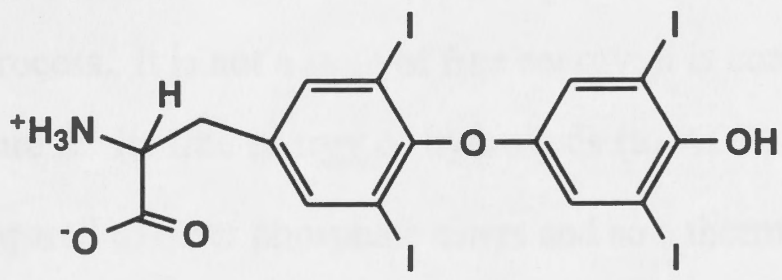
### *Incorporation into Macromolecules and other Functions*

The best known function of α-amino acids is their use as the 'building blocks' from which proteins are synthesised. However, only a small fraction of the many different α-amino acids perform this function; the majority perform other, equally vital roles. Some are incorporated into purines and pyrimidines (the bases in nucleotides and nucleic acids) or into secondary metabolites having properties such as antimicrobial activity.[2] Others are mediators in a variety of biochemical processes such as neurotransmission, immune response and metabolism. For example, thyroxine (**8**), a derivative of tyrosine, is a hormone which stimulates vertebrate metabolism.[3] Other derivatives such as citrulline, **9**, and homocysteine, **10**, are important intermediates in metabolic processes.[3]

### *Asymmetric Syntheses*

When synthesising an organic compound, particularly for a biochemical purpose such as a drug, insecticide or food additive, it is desirable to generate only the one, active, stereoisomer. This means either synthesising a mixture of isomers and then isolating the active isomer from the mixture or, more elegantly, synthesising only the active isomer ('asymmetric synthesis').



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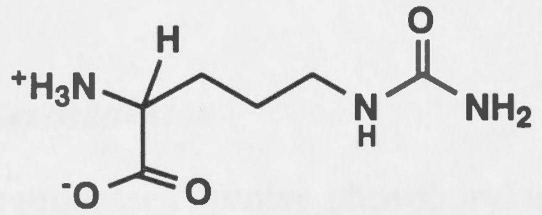

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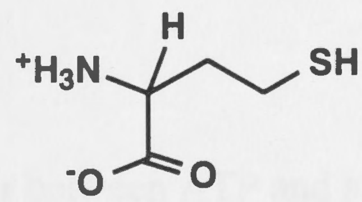

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Optically active amino acids have become increasingly important as both substrates and targets in organic asymmetric syntheses.[4] Recent publications describe highly stereoselective syntheses which use optically pure amino acids as substrates.[4a-e] However, most procedures for obtaining optically pure compounds still require resolution of mixtures of isomers.[5]

## *BIOCHEMISTRY OF PHOSPHATE ESTERS*

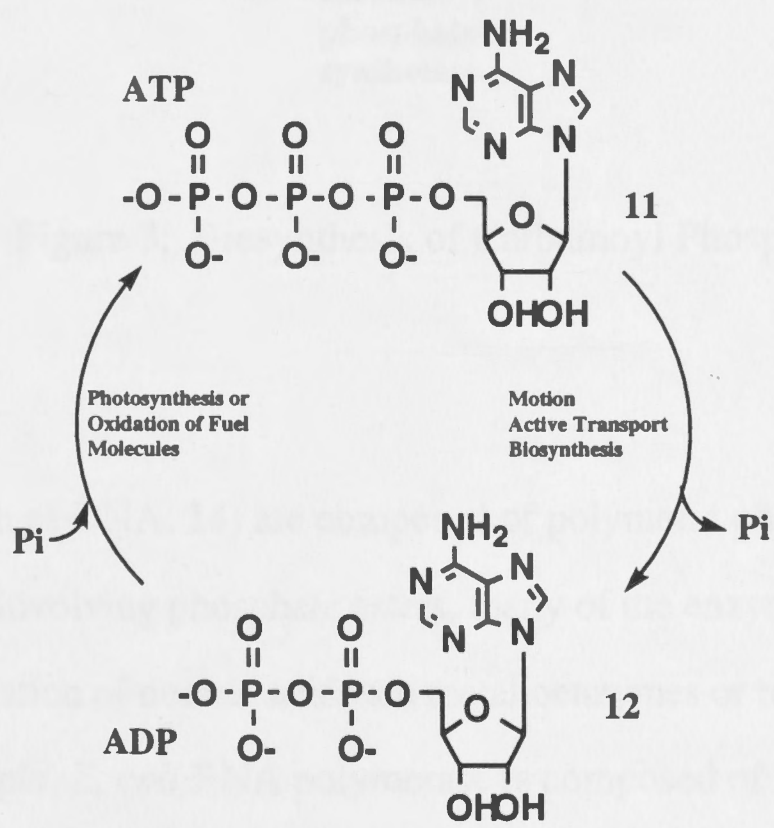


Figure 2: The ATP-ADP cycle, the fundamental mode of biochemical energy transfer.[1]

### *Biological Energy Transfer*

Adenosine triphosphate (ATP), **11**, is the most important source of energy in virtually every cellular process. It is not a store of free energy; it is constantly being formed and consumed, Figure 2. Its free energy of hydrolysis (to ADP, **12**) is high ($\Delta G^{o\prime}$ = -7.3 kcal $mol^{-1}$) compared to other phosphate esters and so a thermodynamically unfavourable reaction can be made more favourable by coupling hydrolysis of ATP to it.

### *Metabolic Intermediates*

Many metabolic processes involve phosphoryl transfer between ATP and an intermediate. For example, during glycolysis all intermediates between glucose (the first component of the cycle) and pyruvate (the last) are phosphorylated.[1] Carbamoyl phosphate (**13**), an important intermediate in the synthesis and degradation of amino acids and nucleotides, is formed from ammonia, bicarbonate and ATP.[1] Phosphorylation of some enzymes is a way of regulating some metabolic pathways. For example, skeletal muscle phosphorylase, which catalyses the phospholyic cleavage of glycogen into glucose-6-phosphate, is rendered active by phosphorylation of serine-14.[1]

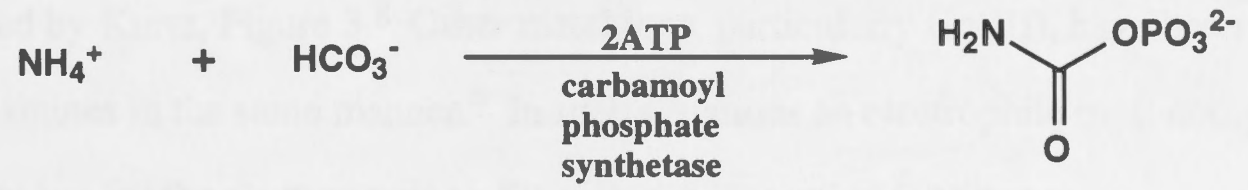


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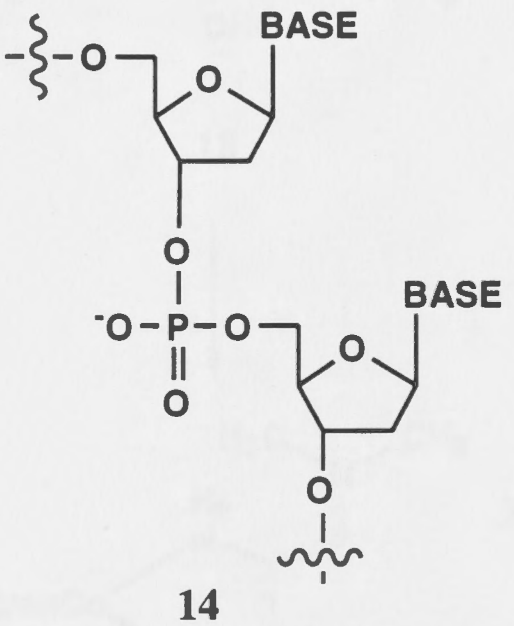
Figure 3: Biosynthesis of Carbamoyl Phosphate

### *Nucleic Acids*

All nucleic acids (such as DNA, **14**) are composed of polymeric phosphodiester units. As with all processes involving phosphate esters, many of the enzymes involved in the replication and degradation of nucleic acids are metalloenzymes or require metal ion cofactors.[6] For example, *E. coli* RNA polymerase is composed of four subunits: $\alpha\beta\beta'\sigma$. The $\beta'$ subunit includes two Zn(II) ions and the enzyme also requires the presence of Mg(II).[3,7] It is most likely that models of biochemical reactions involving phosphate esters (such as hydrolysis of DNA) will require metal complexes to achieve the same modes and levels of activity as the original enzymes.



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## Reactions of Coordinated Ligands

### *EFFECT OF METAL ION ON COORDINATED LIGANDS*

#### *Protection of Functional Groups*

A metal (Cu(II)) ion's ability to protect a coordinated amine from reaction was first described by Kurtz, Figure 3.[8] Other metal ions, particularly Co(III), have been used to protect amines in the same manner.[9] In such situations an electrophile must compete with the metal ion for the electron pair on the nitrogen if reaction is to occur.

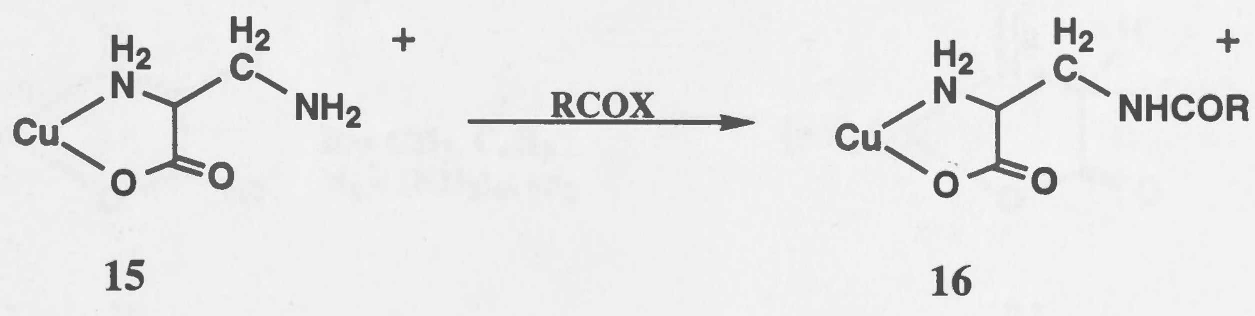
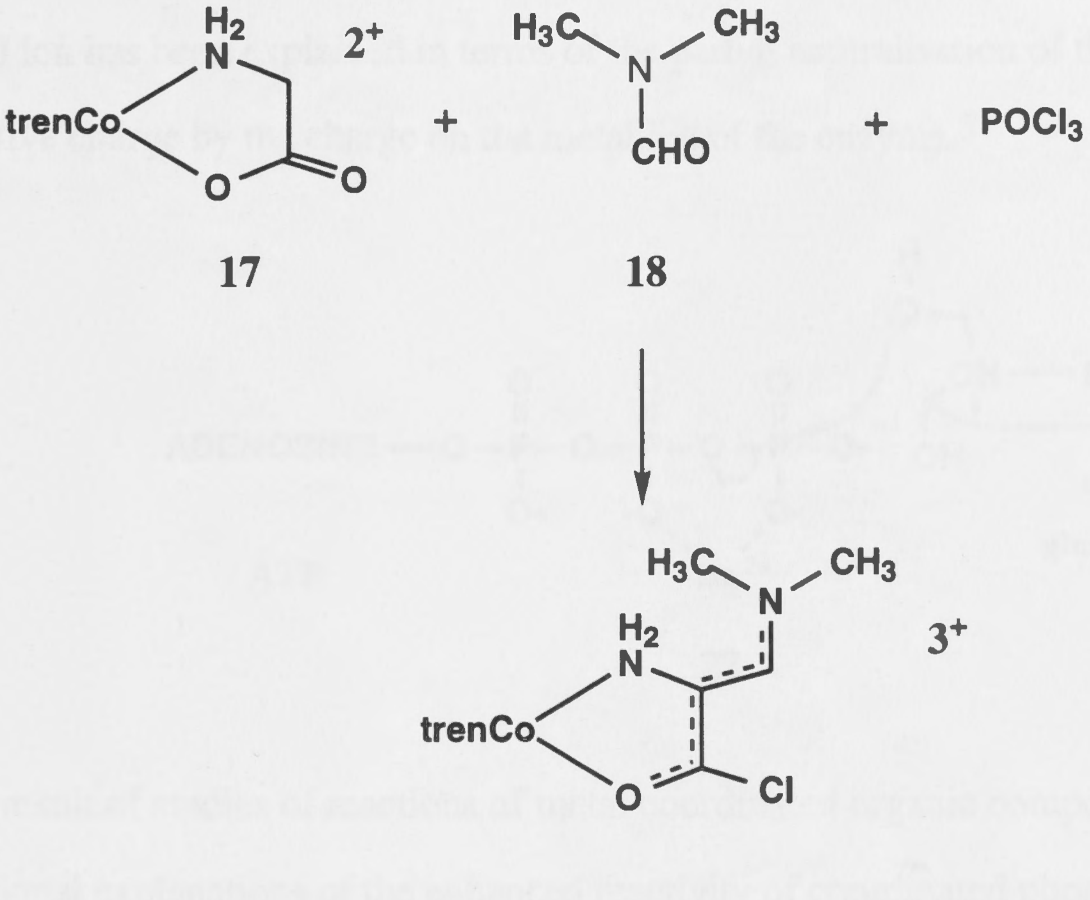


Figure 4: Protection of amine from reaction by coordination to Cu(II)

Coordinated carboxyl moieties are also protected from reaction by coordination to a metal ion. Exceptions include esterification by $SOCl_2$ in alcohols[10] and reaction with the Vilsmeier-Haack reagent ($POCl_3$/dmf), **19**.[11]

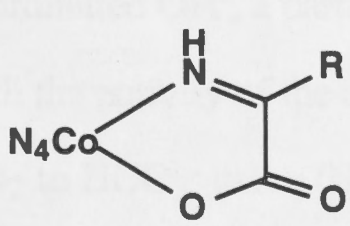


17 18 19

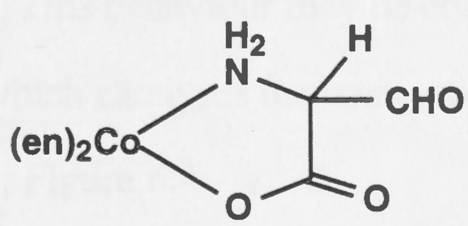
Figure 5: Synthesis and Stabilisation of a Vilsmeier-Haack adduct on Co(III).

### *Stabilisation of Molecules*

Coordination to a metal ion stabilises some species which would otherwise be subject to hydrolysis in aqueous solution. An example of this is the stabilisation of $\alpha$-imino acids (**20**), complexes of which may be readily isolated as solids.[12] Other species include the Vilsmeier-Haack adduct , **19**[11] and formyl glycine, **21**.[13]



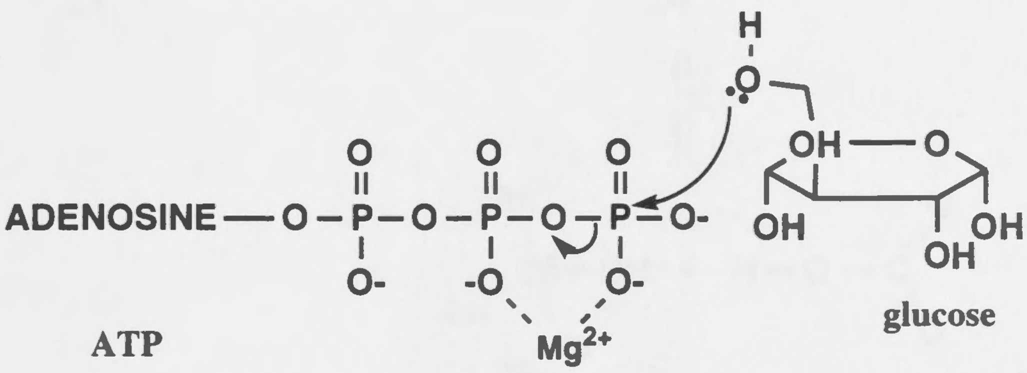
$R = CH_3, C_6H_5$
$N_4 = (NH_3)_4, en_2$



20 21

### *Activation of Sites within the Coordinated Ligand*

Biological phosphoryl transfer and hydrolysis reactions are generally catalysed by metalloenzymes. An example is phosphoryl transfer from ATP to glucose by hexokinase, which occurs only in the presence of Mg(II), **22**. The requirement for the metal ion has been explained in terms of the partial neutralisation of the phosphate's negative charge by the charge on the metal ion of the enzyme.[3]




**22**

As a result of studies of reactions of metal coordinated organic compounds some additional explanations of the enhanced reactivity of coordinated phosphates and other molecules have been advanced . Besides partial neutralisation of negative charge,[14] a metal ion has been found to have an electron withdrawing effect on the ligand, leading to an increase in electrophilicity[14] or an increase in Brønsted acidity[15] of sites in the ligand.

For example, it has been demonstrated that, on coordination to Co(III), the $pK_a$ of ligands such as $H_2O$ decrease (ie $H_2O$ becomes more acidic upon coordination). The $pK_a$ of $H_2O$ in $[(NH_3)_5Co(H_2O)]^{3+}$ is 6.4 compared to that of the free ligand, 15.5.[3,16c] This means that, even at or below neutral pH, there will be significant concentrations of coordinated $OH^-$, a particularly effective nucleophile. This behaviour may be equated with the activity of the enzyme carbonic anhydrase, which catalyses the conversion of $CO_2$ to $HCO_3^-$ using $OH^-$ coordinated to a Zn(II) ion, Figure 6.[3]

The electron withdrawing effect of a metal ion will cause the phosphorus atom of a phosphate ester to become more electrophilic upon coordination and intramolecular hydrolysis of phosphate esters by coordinated $OH^-$ has been demonstrated to be efficient at neutral pH, **26**.[17-20]

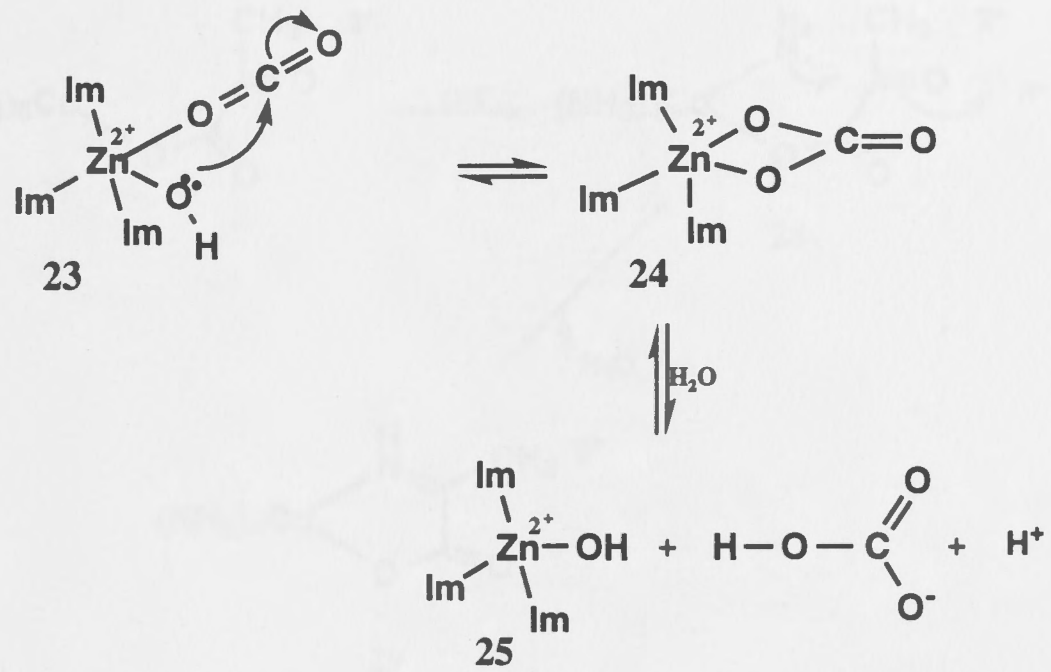
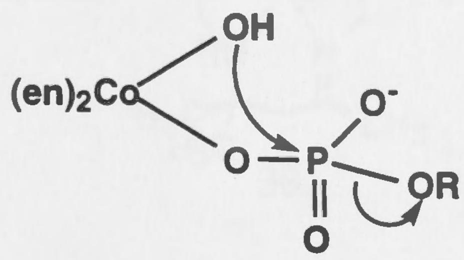


Figure 6: Conversion of $CO_2$ to $HCO_3^-$ by carbonic anhydrase.
Im = imidazole (from a histidine side chain).

Synthesis of α-imino acid complexes of Co(III) can take place by intramolecular condensation of coordinated amido ion ($NH_2^-$) on the β-carbon of an α-keto acid coordinated *cis* to the amido ion, Figure 7.[21] Ammonia becomes more acidic on coordination to Co(III) and deprotonates readily in base, generating an amido ion, a good nucleophile, **27**. This species then attacks the electrophilic β-carbon, generating the carbinolamine **28**, which swiftly eliminates water and rearranges to **29**, the α-imino acid complex. The α-imino acid complex is itself activated by coordination to Co(III). The reactions of coordinated α-imino acids are described in more detail below but the point is made here that activation of α-amino and imino acids is achieved through the combined effect of coordination of the nitrogen of the imine or amine and of the carboxylate (forming a chelate) to the metal centre.



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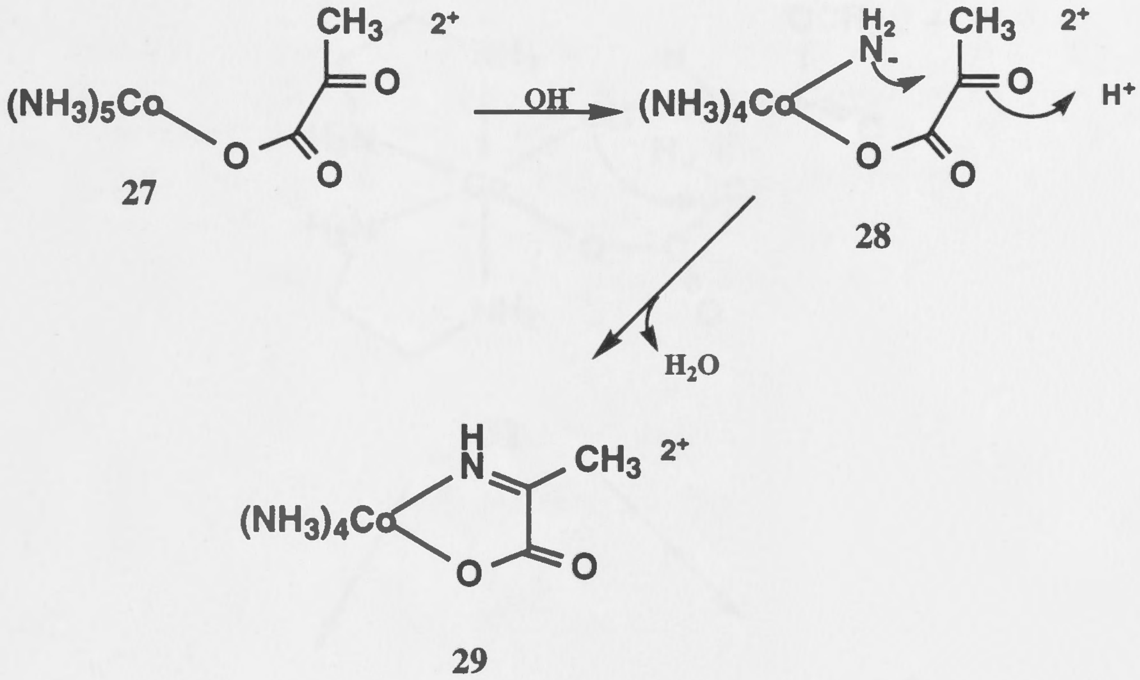


Figure 7: Synthesis of Co(III) complexes of α-imino acids.

***The Template Effect***

The so called 'template effect' is the term given to the way in which the geometry of coordination to a metal ion directs a ligand's reactivity. In an early example, the Curtis synthesis involved the reaction of $[(en)_3Ni]^{2+}$ with acetone, to produce a mixture of isomers **30a** and **30b**.[22] Substitution inert metal ions such as Co(III) are particularly useful for controlling the outcomes of a reaction in this way. For example, if a complex (**31**) involving monodentate methyl maleate is mixed in 0.1 M NaOH, then coordinated $OH^-$ will attack the olefin. The olefin may be oriented in two ways (**32a** and **32b**).

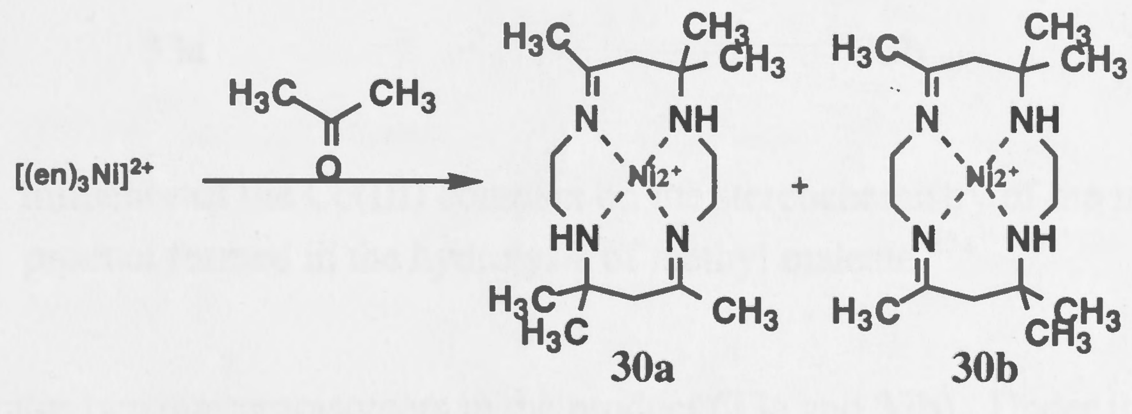


Figure 8: Metal Ion Template Effect: the Curtis synthesis.[22]

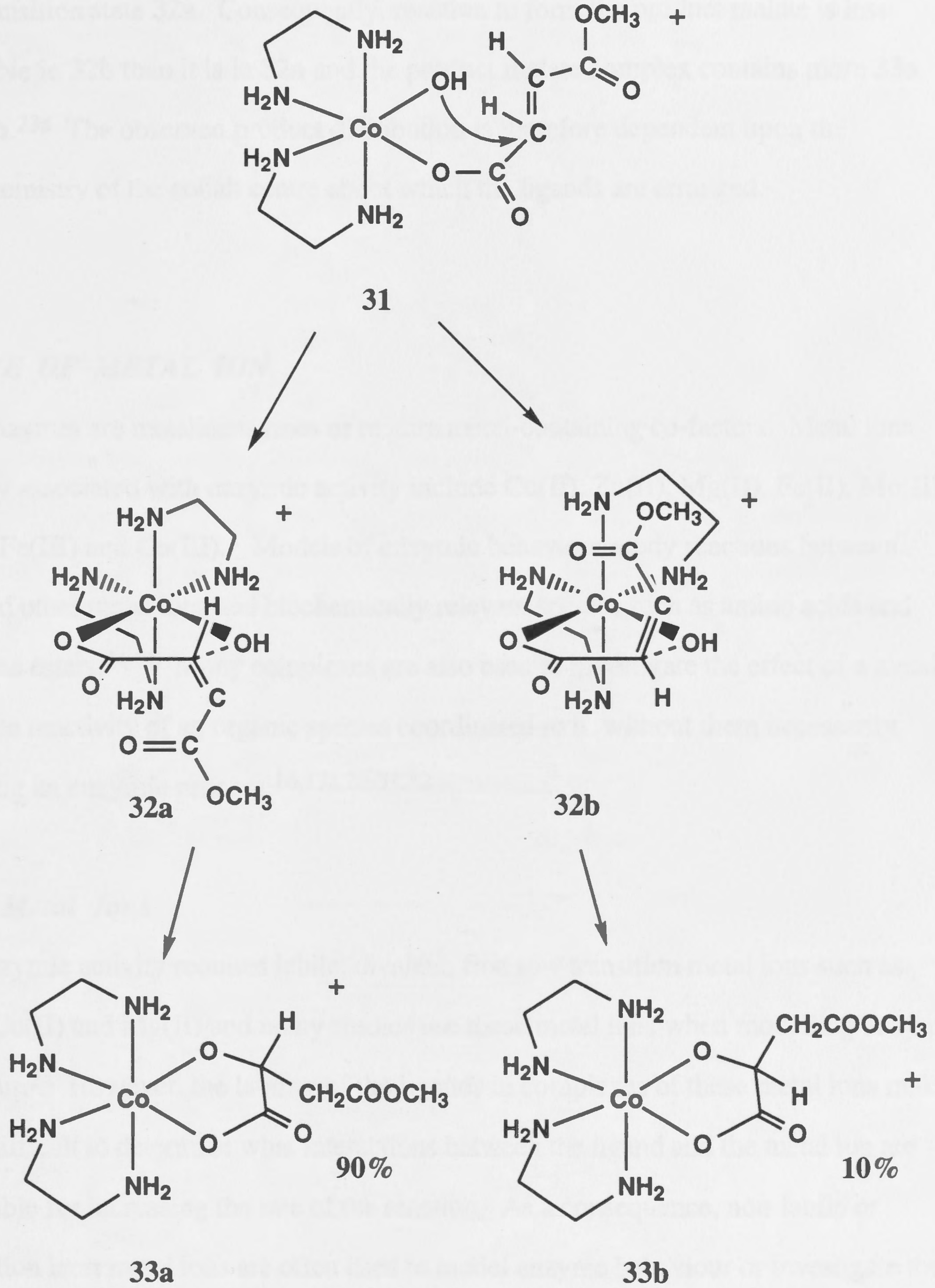



Figure 9: Influence of the Co(III) complex on the stereochemistry of the malate product formed in the hydrolysis of methyl maleate.[23a]

This generates two diastereoisomers in the product (**33a** and **33b**). Under the conditions of the experiment these were formed in the ratio of 9:1 respectively. An explanation for this ratio is that in the transition state **32b** there are conceivably a greater number of non-bonded interactions between the olefin and the adjacent en coligand than there is in the other transition state **32a**. Consequently, reaction to form the product malate is less favourable in **32b** than it is in **32a** and the product malate complex contains more **33a** than **33b**.[23a] The observed product distribution is therefore dependent upon the stereochemistry of the cobalt centre about which the ligands are arranged.

## *CHOICE OF METAL ION*

Many enzymes are metalloenzymes or require metal-containing co-factors. Metal ions regularly associated with enzymic activity include Cu(II), Zn(II), Mg(II), Fe(II), Mo(II), Mn(II), Fe(III) and Co(III).[3] Models of enzymic behaviour study reactions between these and other metal ions and biochemically relevant species such as amino acids and phosphate esters.[24-30] Many complexes are also used to investigate the effect of a metal ion on the reactivity of an organic species coordinated to it, without them necessarily modelling an enzymic process.[16,17a,23,31,32]

### *Labile Metal Ions*

Most enzymic activity requires labile, divalent, first row transition metal ions such as Zn(II), Cu(II) and Mg(II) and many studies use these metal ions when modelling enzyme behaviour.[24] However, the lability of the ligands in complexes of these metal ions makes it very difficult to determine what interactions between the ligand and the metal ion are responsible for increasing the rate of the reaction. As a consequence, non-labile or substitution inert metal ions are often used to model enzyme behaviour or investigate the metal ion promoted reactions of organic ligands.

### *Non-Labile Metal Ions*

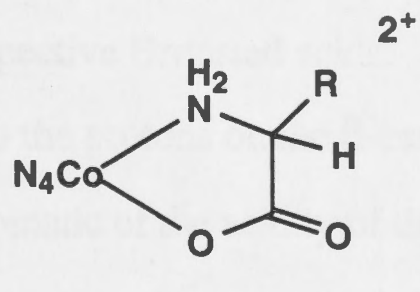
Metal ions such as Co(III), Cr(III), Ir(III) and Rh(III) have the advantage of holding ligands in kinetically inert complexes having a definable geometry.[16,17,33,34] This means that it is possible to isolate intermediates and determine the pathway by which a reaction occurs. It is also possible to control the stereochemistry of such complexes and so induce stereoselectivity in the reaction.

Reactions involving complexes of diamagnetic metal ions have an added advantage in that they may be monitored using nmr spectrometry. For this reason (and because it is relatively inexpensive and provides coloured complexes) Co(III) is a particularly popular metal centre for use in studying reactions of coordinated ligands. At the end of the reaction inert Co(III) can be reduced to labile Co(II) and the new organic product isolated.

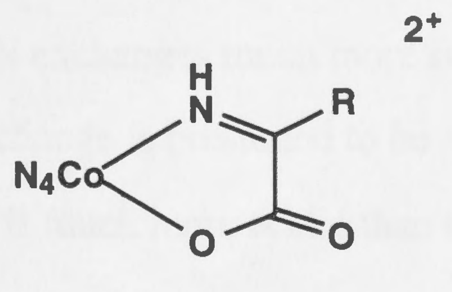
## Reactions of α-Amino and Imino Acids Coordinated to Co(III).

### *GENERAL STRUCTURE OF THE COMPLEXES*

The complexes discussed below have the general composition **34** (α-amino acid complexes) and **35** (α-imino acid complexes) There are four sites in these complexes that are activated by their coordination to Co(III): the imine-N of the imino acid complex, the α- and β- carbons of the amino and imino acids and the amines/ammines of the coligands. The amine moiety of the amino acid and the carboxyl group of both the amino and imino acids are largely protected from reaction by coordination to Co(III).



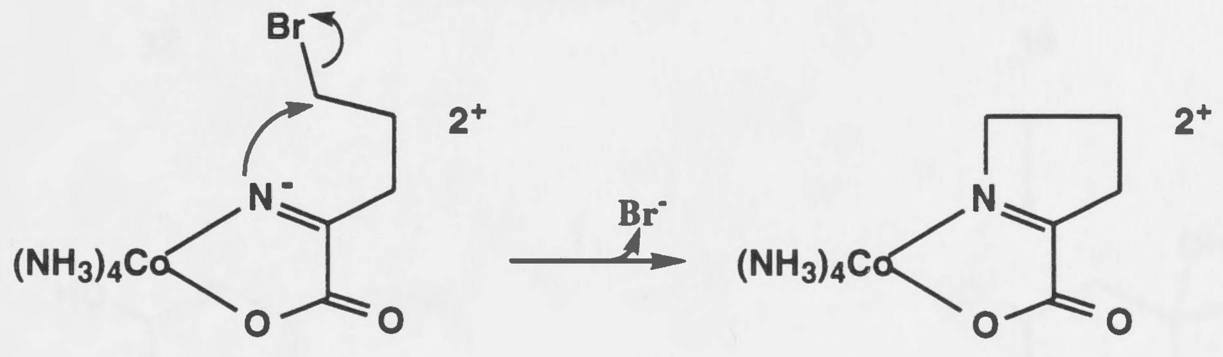
**34**



**35**

### *REACTION AT IMINE-N*

The Co(III)-coordinated imine is relatively acidic, the $pK_a$ of the proton is in the range 9.5 to 10.5 depending on the R substituent.[21] Some imine-N deprotonated complexes are also stable enough that they may be isolated from solution.[21,33] Deprotonated imino acid complexes readily undergo intermolecular reactions with alkylating reagents such as methyl iodide, allyl bromide and benzyl bromide,[21,33] whilst an intramolecular reaction involving deprotonated imine-N occurs when the nucleophile displaces bromide ion from the side chain of **36** in the synthesis of pyrroline, **37**.[35]




**36** **37**

Figure 10: Reaction at Imine-N: intramolecular cyclisation by elimination of $Br^-$ to form pyrroline.

The imine-N nucleophile has also been reacted with the carbonyl moiety of ketones and esters and with alkenes and alkynes.[33,36] For example, reaction between sodium diethyloxaloacetate and **38** leads to the substituted pyridine ligand **4**, Figure 10.[33] In this instance two sites, the imine-N and β-carbon of the complex, are involved in the synthesis of the product. The problem of which anion ($=N^-$ or $CH_2^-$) adds first to the electrophile may be addressed by consideration of the two species as conjugate bases of the respective Brønsted acids. The proton on the imine-N exchanges much more swiftly than do the protons on the β-carbon.[21b] If the rate of exchange is presumed to be symptomatic of the acidity of the site[33] then the imine-N is much more acidic than the β-carbon and will be the predominant nucleophile in the reaction.[36] Consequently, the intermediates will be imine-N substituted.

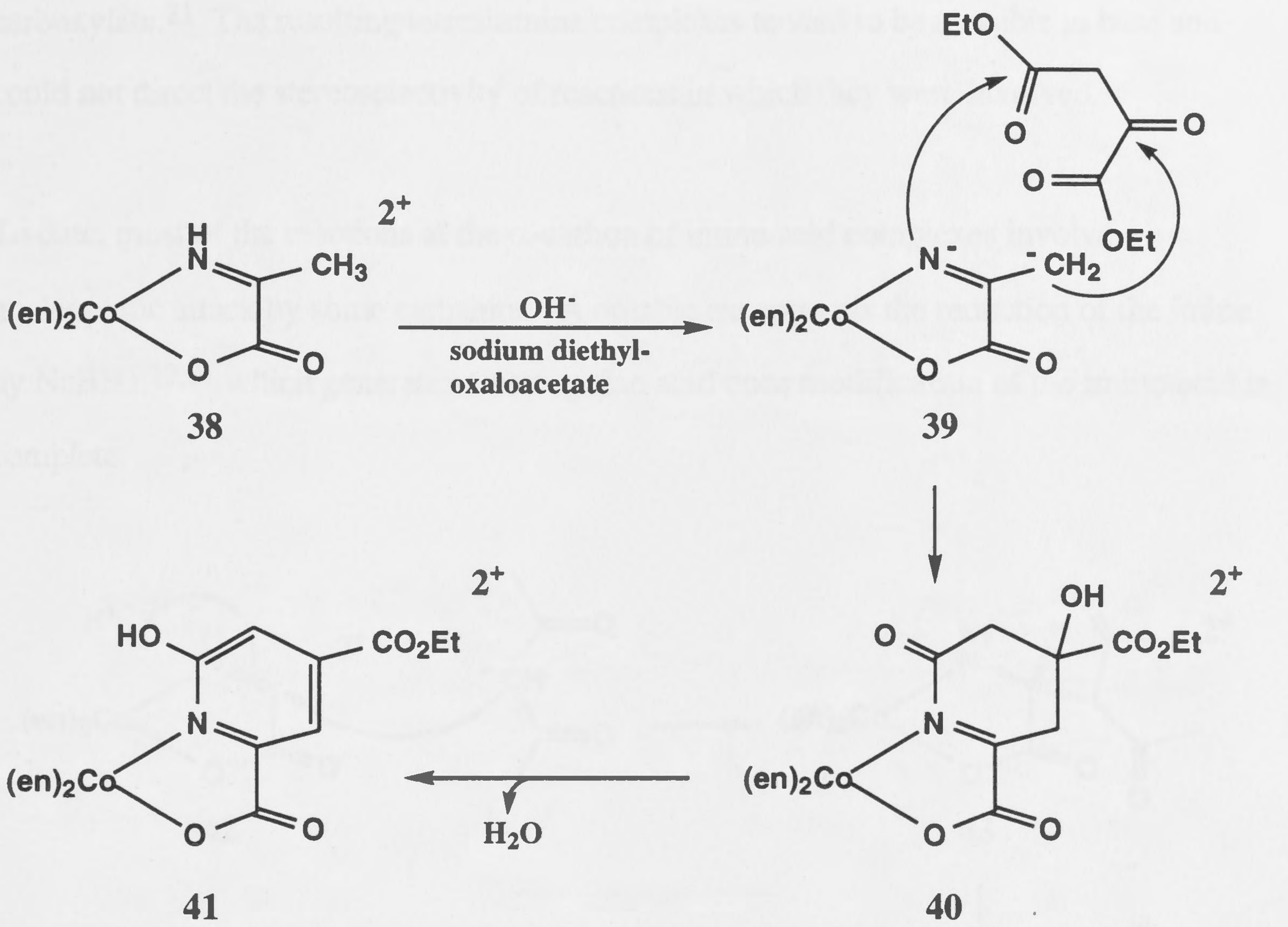



Figure 11: Reaction at imine-N and β-carbon to yield a substituted pyridine ligand.[33]

## *REACTION AT α-CARBON*

Activation of the α-carbon of coordinated amino acids (in the form of increased acidity of the protons on the atom) was demonstrated by Busch and Williams.[15c] Further investigation of the reactivities of such complexes found that the rate of exchange of the protons in $OD^-$ solution was comparable to the rate of racemisation, implicating an α-carbanion intermediate in the exchange.[15a,37]

A wide variety of α-amino acid complexes have also been oxidised by the electrophilic reagent SOCl to form the corresponding α-imino acid complexes.[38] Prior to the development of this reaction strategy, most α-imino acid complexes were obtained by intramolecular attack of coordinated ammonia on the corresponding monodentate α-keto carboxylate.[21] The resulting tetraammine complexes tended to be unstable in base and could not direct the stereoselectivity of reactions in which they were involved.

To date, most of the reactions at the α-carbon of imino acid complexes involve nucleophilic attack by some carbanion. A notable exception is the reduction of the imine by $NaBH_4$,[12,21] which generates a new amino acid once modification of the imino acid is complete.

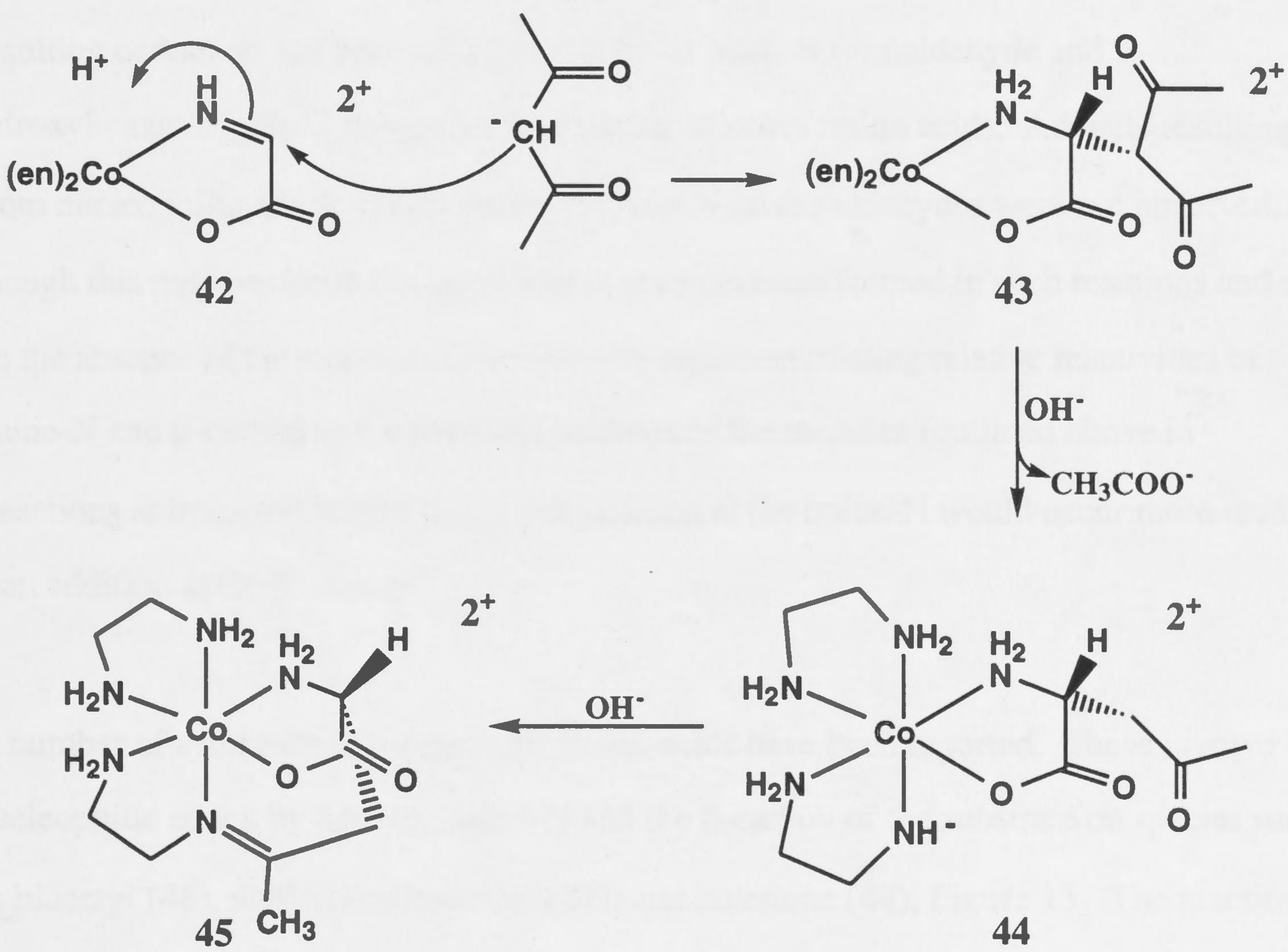



Figure 12: Reaction of acetyl acetone with the α-carbon and coligand-N of $[en_2Co(gly\text{-}im)]^{2+}$.[41]

Additions at the α-carbon include cyanide ion,[12a,39] acetyl acetone,[36,40] nitromethane[12,33] and ethyl cyanoacetate.[33] In most instances, the incoming nuclephile can (and does) react at two sites on the imino acid complex; the electrophilic α-carbon of the imino acid and nucleophilic amines on the coligands. As with reactions involving the imine-N, the question of which intermediate forms, or which reaction takes place first, should be addressed. In most instances no intermediates were detected; however, a study involving the addition of acetyl acetone to $[(en)_2Co(gly\text{-}im)]^{2+}$ isolated and identified the adduct **44** in the mixture of products.[41] Its existence implies that, at least in this instance, addition at the α-carbon precedes that at the coligands.

### *REACTION AT β-CARBON*

The β-carbon of coordinated α-imino acids is capable of functioning as a nucleophile in basic conditions. Protons on the carbon atom undergo exchange in $OD^-$ solutions and the resulting carbanion has been added to aldehydes such as formaldehyde and 3-hydroxybenzaldehyde[12] to synthesis a number of novel imino acids. Adducts resulting from nucleophilic attack of deprotonated imine-N on the aldehydes were not observed, though this may be due to the instability of intermediates formed in such reactions and not on the absence of the reaction. Certainly, the argument relating relative reactivities of imine-N and β-carbon to the Brønsted acidities of the moieties (outlined above in Reactions at Imine-N) would imply that addition at the imine-N would occur more readily than addition at the β-carbon.

A number of syntheses of heterocyclic imino acids have been reported. These involve nucleophilic attack by both the imine-N and the β-carbon of the substrate on species such as biacetyl (**46**), diethyl oxaloacetate (**47**), and butenone (**48**), Figure 13. The reaction mechanisms are described in terms of sequential attack on the electrophile, first by imine-N, then by the β-carbon.[33,36]

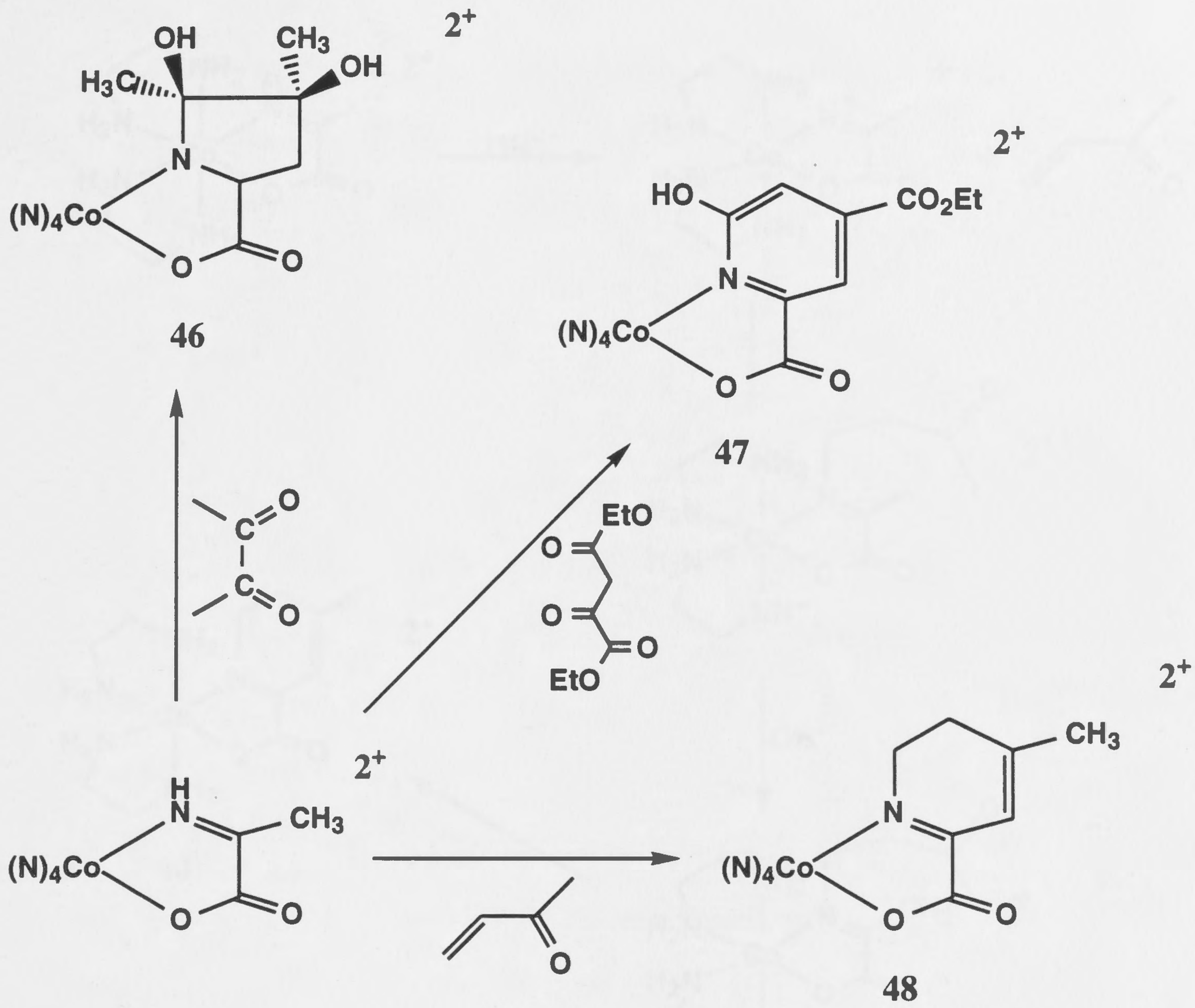



Figure 13: Reactions at the β-carbon of coordinated α-imino acids which results in heterocyclic amino acids.

### *REACTION AT NITROGEN OF THE COLIGANDS*

Amine moieties in the coligands of α-imino acid complexes often compete with the β-carbon for an electrophile attached to the imine-N or α-carbon.[12,33,39-41] If proton exchange rates are again presumed to be indicative of Brønsted acidity[33] then the amines/ammines of the coligands are more acidic than the methyl group will be more likely to react with the available electrophile as a consequence.[36] For example, butenone reacts with $[(en)_2Co(ala\text{-}im)]^{2+}$ to produce a mixture of products, Figure 14. Most of the substrate has reacted at the imine-N and coligand-N (**49** and **50**) with butenone and the heterocyclic product (**48**), formed by reaction of butenone with the imine-N and β-carbon, is only a minor product.[33]

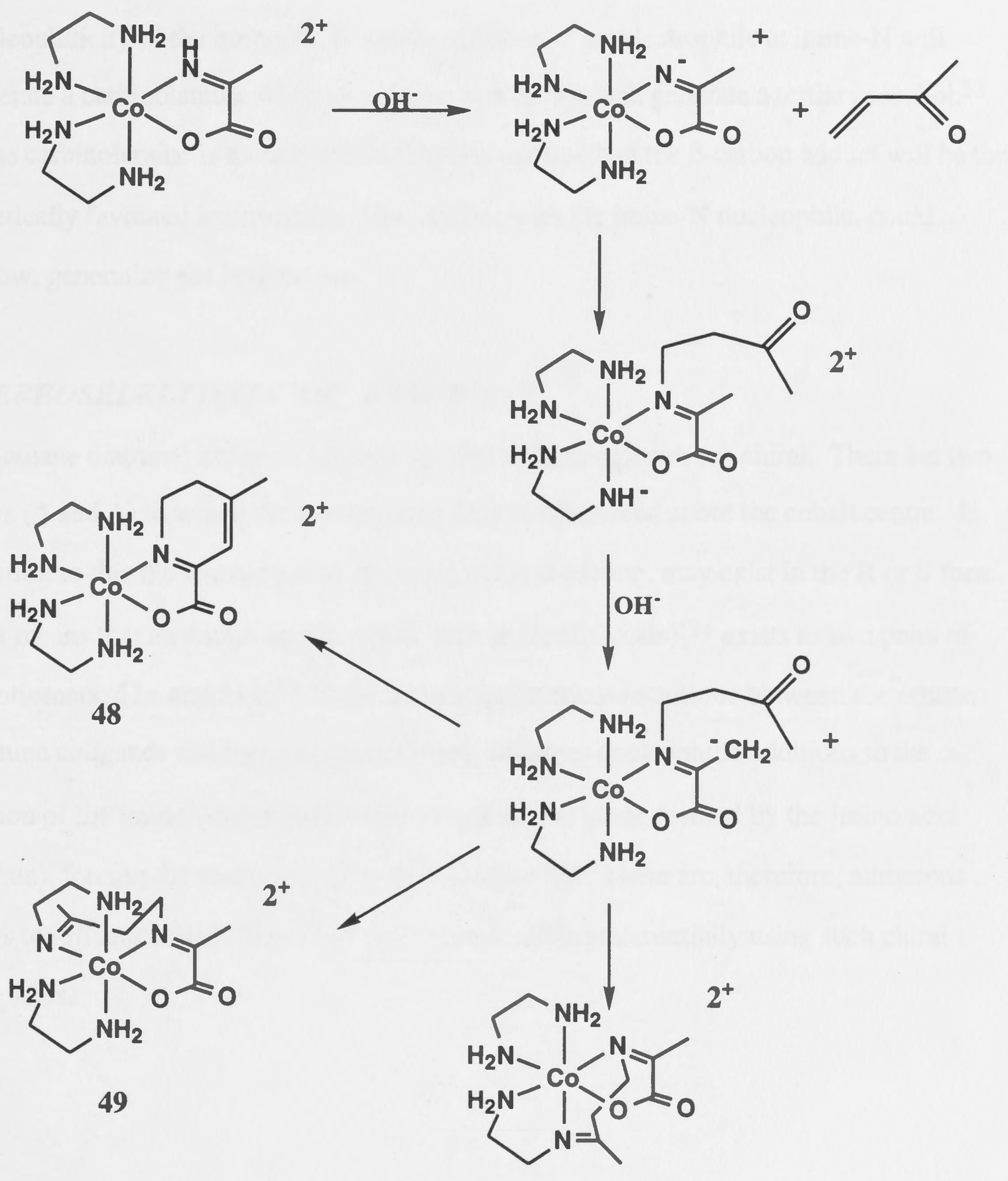
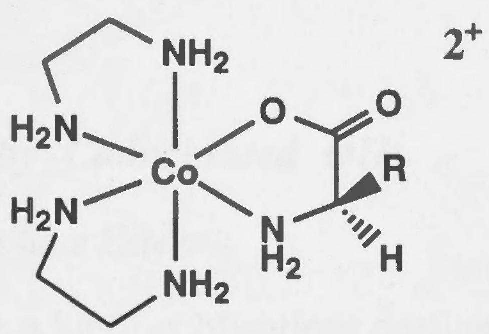



Figure 14: Reaction of butenone at multiple sites within $[en_2Co(ala\text{-}im)]^{2+}$.[33]

In most instances the coligand-N has proved to be a better nucleophile than the β-carbon. However, in some instances, a heterocyclic imino acid is the predominant product. For example, diethyl oxaloacetate reacts with $[(NH_3)_4Co(ala\text{-}im)]^{2+}$ and $[(en)_2Co(ala\text{-}im)]^{2+}$ to yield the substituted pyridine noted earlier, Figure 11.[33,36] In such instances the product may result from an intermediate formed by reaction of the β-carbon with the electrophile instead of resulting from addition first at the imine-N. This might appear counter to the argument for increased Brønsted acidity and hence increased nucleophilicity of the imine-N. However, addition of an electrophile at imine-N will generate a carbinolamine whereas addition at β-carbon will generate a tertiary alcohol.[33] If the carbinolamine is more unstable than the alcohol then the β-carbon adduct will be the kinetically favoured intermediate. Cyclisation, with the imine-N nucleophile, could follow, generating the heterocycle.

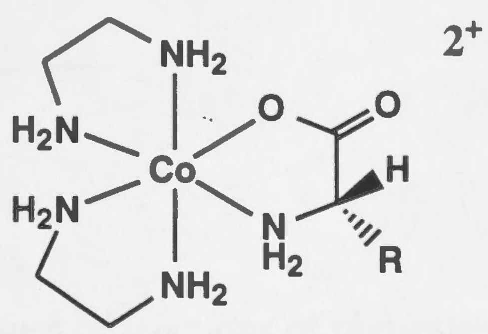
### *STEREOSELECTIVITY OF REACTIONS*

Bis(ethane diamine) chelated amino or imino acid cobalt(III) complexes are chiral. There are two ways (Δ and Λ) in which the chelate rings may be orientated about the cobalt centre. In addition to this the α-amino acid, having a chiral α-carbon, may exist in the R or S form. This means that an amino acid complex such as $[(en)_2Co(ala)]^{2+}$ exists as two pairs of enantiomers, **51a** and **51b**.[42] Non-bonding and steric interactions between the ethane diamine coligands and the α-imino acid may influence nucleophilic additions to the α-carbon of the imino acid (from 'above' or 'below' the plane formed by the imino acid chelate), forcing the reaction to be stereoselective.[23a] There are, therefore, numerous ways to influence the formation of chiral amino acids preferentially using such chiral templates.



ΛR, ΔS

**51a**



ΛS, ΔR

**51b**

## Reactions of Phosphate Esters Coordinated to Co(III)

To date, the vast majority of studies have involved hydrolysis of phosphate esters or polyphosphates when coordinated to Co(III). There have been very few reports of syntheses of phosphate esters or polyphosphates. As noted previously, coordination of a phosphate species to a metal centre will decrease the negative charge on the ligand and make the phosphorus atom more electrophilic[17] Both effects will tend to increase the rate of reaction with nucleophiles. Phosphate ligands tend to be rather more labile toward dissociation than imino and amino acid ligands and this can be a problem in these studies.

### *INTERMOLECULAR ATTACK BY NUCLEOPHILES*

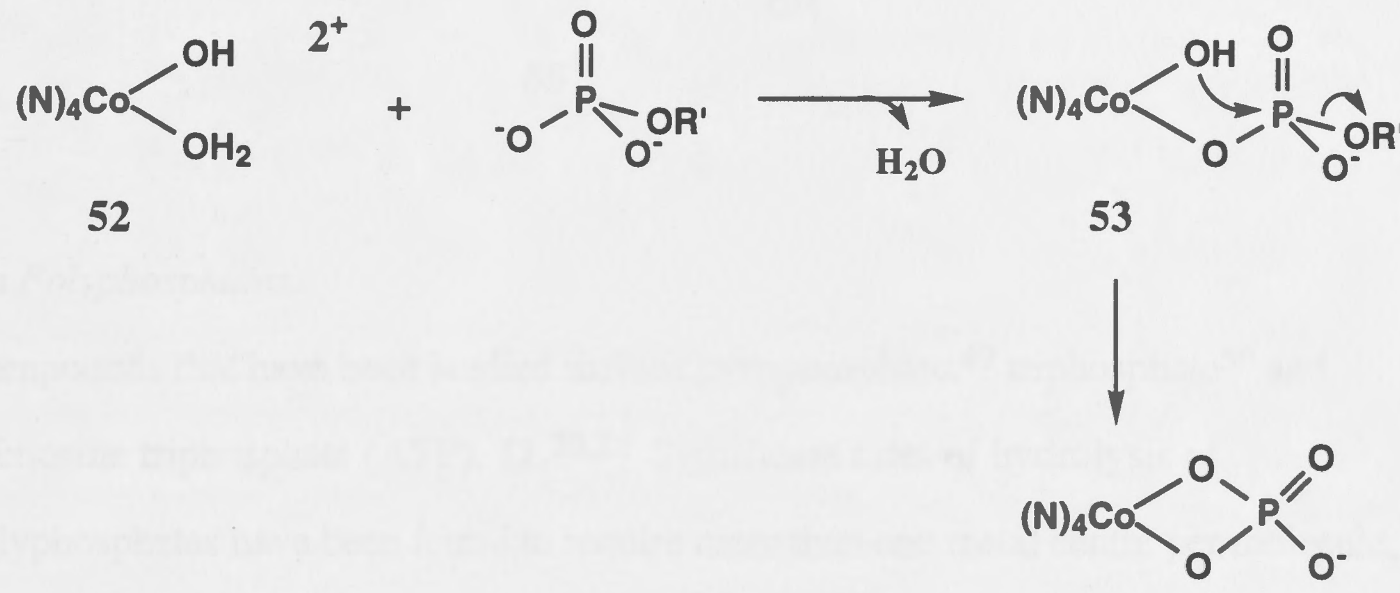
Examples of these types of reactions include the hydrolysis of $[(NH_3)_5Co(TMP)]^{3+}$, $[(NH_3)_5Rh(TMP)]^{3+}$, and$[(NH_3)_5Ir(TMP)]^{3+}$ (TMP = trimethyl phosphate).[43-45] In the rhodium and iridium complexes, $OH^-$ attacks the phosphorus centre, causing cleavage of a P-O bond. In the case of the cobalt complex, the rate of hydrolysis is less competitive than dissociation of TMP from Co(III). If 'softer' nucleophiles such as $SCN^-$, $I^-$ and $S_2O_3^{2-}$ are substituted for $OH^-$ then hydrolysis of $[(NH_3)_5Co(TMP)]^{3+}$ occurs, via C-O cleavage.[46]

### *INTRAMOLECULAR ATTACK BY NUCLEOPHILES*

#### *Attack by Coordinated OH⁻*

*On Phosphate Esters*

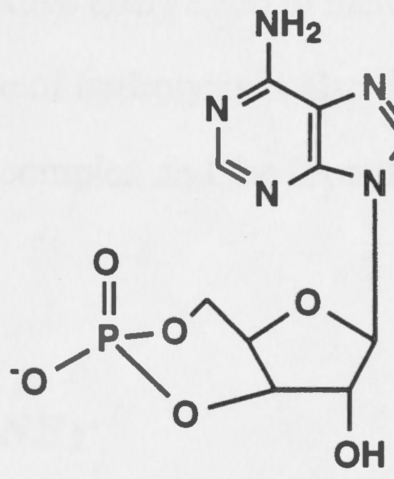
There are a lot of publications dealing with these types of reactions of phosphate esters.[17] These mostly deal with reactions between $[N_4Co(OH)(H_2O)]^{2+}$ and $OPO_2(OR)^{2-}$ or $OPO(OR)(OR')^-$, Figure 15. The cobalt complex is composed of a potent nucleophile, $OH^-$, *cis* to a labile ligand, $H_2O$, which is readily replaced by the phosphate ester. Hydrolysis of the phosphate ester then occurs at physiological pH through intramolecular attack of $OH^-$ on the phosphate centre, **53**. The tetraamine coligand(s) are quite substitutionally inert so the complex remains fairly stable over the time of the hydrolysis reaction. The rate of hydrolysis has been found to follow the rate of exchange of phosphate ester for coordinated water, indicating that the rate limiting step in the reaction is probably the substitution reaction, to form **53**.[17]




**52** **53** **54**

Figure 15: Intramolecular hydrolysis of phosphate esters by coordinated $OH^-$.

Cyclic adenosine monophosphate (cAMP), **55**, is involved in the action of a number of different hormones such as adrenalin.[3] When no longer required it is hydrolysed by a phosphodiesterase, the activity of which is dependent upon Mg(II). There have been a number of studies of the hydrolysis of c-AMP by complexes of the form $[N_4Co(OH)(OH_2)]^{2+}$. The complex $[\text{trienCo}(OH)(OH_2)]^{2+}$ was found to increase the rate of hydrolysis of c-AMP by a factor of about $10^8$ over the hydrolysis of the free ligand at pH 7 and 50 $^oC$.[47] Some more recent work examined the ratio of 5'- to 3'-AMP produced upon hydrolysis of cAMP.[48] The complex $[\text{trienCo}(OH)(OH_2)]^{2+}$ was found to yield predominantly 3'-AMP whilst $[(tn)_2Co(OH)(OH_2)]^{2+}$ was found to yield predominantly the 5'-AMP. This can be interpreted in terms of different steric demands by the complexes when bound to c-AMP and implies the possibility of selective (3'- or 5') cleavage of nucleic acids.



cAMP

**55**

*On Polyphosphates*

Compounds that have been studied include pyrophosphate,[49] triphosphate[50] and adenosine triphosphate (ATP), **11**.[20,26] Significant rates of hydrolysis of polyphosphates have been found to require more than one metal centre per molecule, to

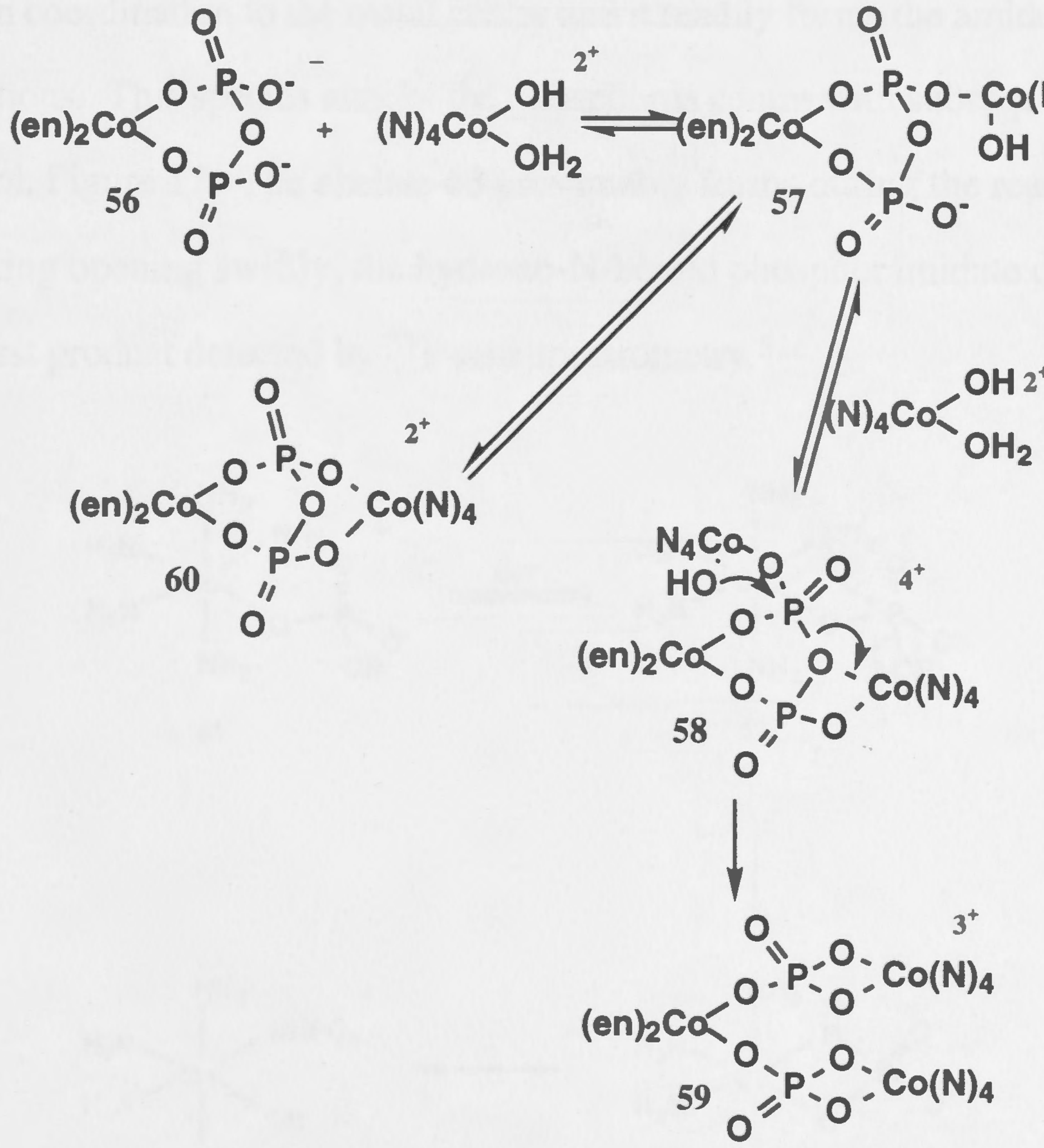


Figure 16: Co(III) promoted hydrolysis of pyrophosphate.[49a]

neutralise the increased negative charge and to increase the electrophilicity of the phosphate centre.[49] The rate of hydrolysis is also dependent upon the rate of exchange of coordinated water on the complex and the formation of unreactive species such as **60**.[49a]

### *Attack by Coordinated $NH_2^-$*

The action of a number of enzymes is controlled by phosphorylation of a histidine residue in the protein chain. These include phosphoglycerate mutase (an enzyme in glycolysis) and E1, a cytoplasmic protein involved in the phosphoenolpyruvate-dependent phosphotransferase system, which engages in the transport of sugars across the cell membrane.[3] These are instances of phosphoryl transfer from oxygen to nitrogen and there are some studies modelling this reaction in the literature.[51] These generally utilise pentammine complexes of a phosphate ester, such as **61**. The acidity of ammonia is increased on coordination to the metal centre and it readily forms the amido ion ($NH_2^-$) in basic conditions. This species attacks the phosphorus centre with subsequent elimination of an alcohol, Figure 17. The chelate **63** presumably forms during the reaction but undergoes ring opening swiftly, the hydroxo-N-bound phosphoramidate complex (**64**) being the first product detected by $^{31}P$ nmr spectrometry.[51c]

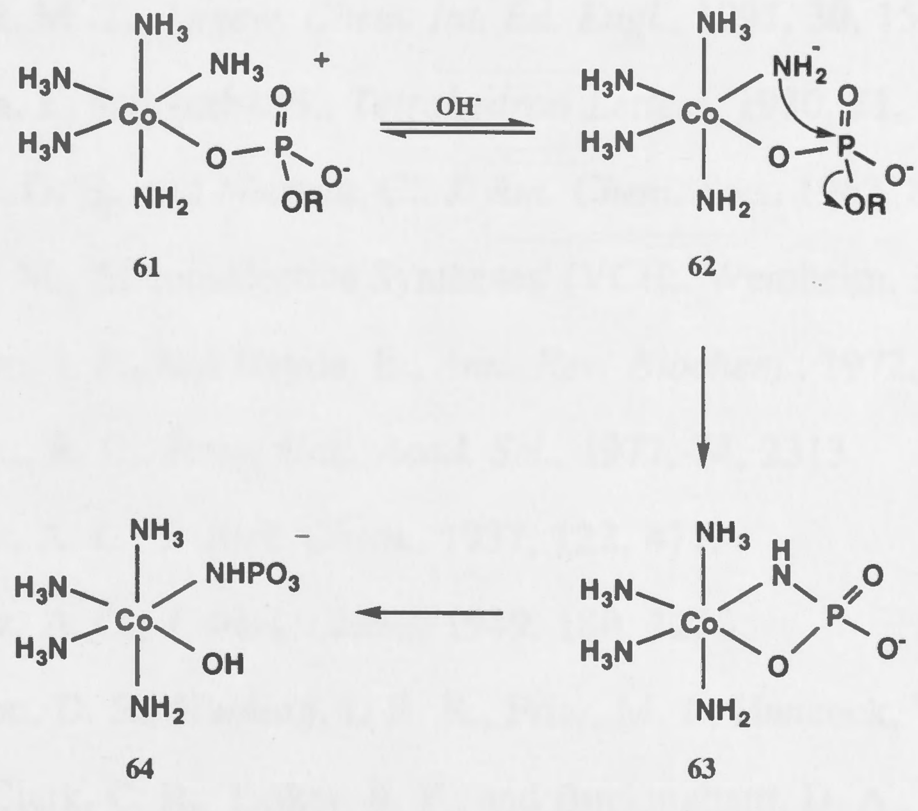



Figure 17: Intramolecular attack of amido ion on phosphate esters.[51c]

This brief survey of reactions of biological substrates such as amino acids and phosphate derivatives gives an indication of the potential for further development of such reactions for synthesis and degradation of such molecules.

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# CHAPTER 2

## Reduction of Co(III) Coordinated α-Imino Acids by Sodium Dithionite

## Introduction

Sodium dithionite, $Na_2S_2O_4$, is an inexpensive, relatively powerful, readily available reducing agent, which is used for a variety of purposes, under a wide range of circumstances. It is used in industry, for the manufacture of various chemicals, and in bleaching and dyeing processes.[1] It is also a popular reducing agent in biochemistry. In particular, it is used to prepare the reduced form of various enzymes,[2] to maintain anaerobic conditions in oxygen sensitive systems and when investigating the kinetics and mechanisms of enzymic processes.[3,4] It is able to reduce some unsaturated organic molecules such as nitrogen containing heterocycles, oximes, nitro compounds, alkenes and carbonyl compounds. It is used in neutral to slightly basic conditions and it was thought to be an effective reagent for reducing chelated imino acids to amino acids.

### *MECHANISM OF THE REDUCTION REACTION*

In general terms, the dithionite ion may reduce a substrate directly, or by first dissociating, to form two sulfinate radicals ($SO_2^{\cdot-}$).

$$S_2O_4^{2-} = 2SO_2^{\cdot-} \qquad \mathbf{I}$$

$$S_2O_4^{2-} + \text{substrate} = \text{products} \qquad \mathbf{II}$$

$$SO_2^{\cdot-} + \text{substrate} = \text{products} \qquad \mathbf{III}$$

Consequently, a reduction reaction involves a two electron process (involving the $S_2O_4^{2-}$ moiety) or a one electron or two sequential one electron process(es) (involving the $SO_2^{\cdot-}$ moiety). In some organic systems the reduction reaction can be described in terms of a Michael type addition, dithionite ion being the nucleophile which adds to an electrophilic carbon of the substrate.[5,6] In systems involving metal complexes or metalloenzymes, the reduction process is usually defined in terms of electron transfer between the reducing species and the metal centre.[7,8,9]

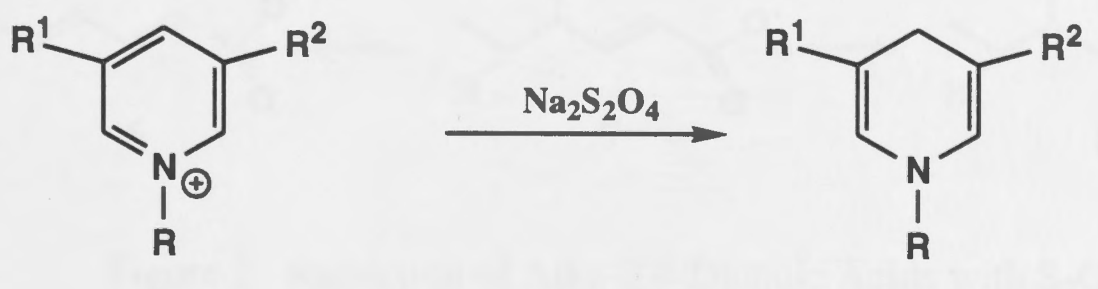
Studies of the kinetics of the reduction of a substrate by dithionite yield pseudo first order rate constants ($k_{obs}$) of the general form: $k_{obs} = a[S_2O_4^{2-}] + b[S_2O_4^{2-}]^{1/2}$, where the terms describe the contribution to the reduction reaction of dithionite ion and sulfinate radical respectively. The sulfinate radical is present in much lower concentrations than dithionite ion. However, it is a much stronger reductant than dithionite ion; reduction potentials (pH 7.0, versus NHE) are -0.66 and -0.18 V for the $SO_2^{\cdot -}/HSO_3^-$ and $S_2O_4^{2-}/HSO_3^-$ couples respectively.[10] Consequently, $SO_2^{\cdot -}$ is very often the predominant reducing species, particularly at low concentrations of $S_2O_4^{2-}$.[1(a),11]

## *SODIUM DITHIONITE AS A REDUCING AGENT*

### *Reactions of $S_2O_4^{2-}$ in organic chemistry*

Sodium dithionite has been a valuable reductant in organic chemistry for quite some time. For example, in 1948 Smith and Schubert reported the synthesis of a number of poly-substituted aminophenols by reduction of the corresponding oximes with dithionite.[12] A survey of the literature reveals that the salt may be used to reduce a wide range of functional groups, including imines, ketones, enones, amides and nitro groups.

*Reactions with derivatives of pyridine and related compounds:* sodium dithionite has been used to reduce substituted pyridine and pyridinium salts, Figure 1.[13-17] Studies of $NAD^+$ and its analogues, particularly nicotinamide derivatives,**1a**, have used dithionite to generate the corresponding dihydropyridine derivatives.[13,16]



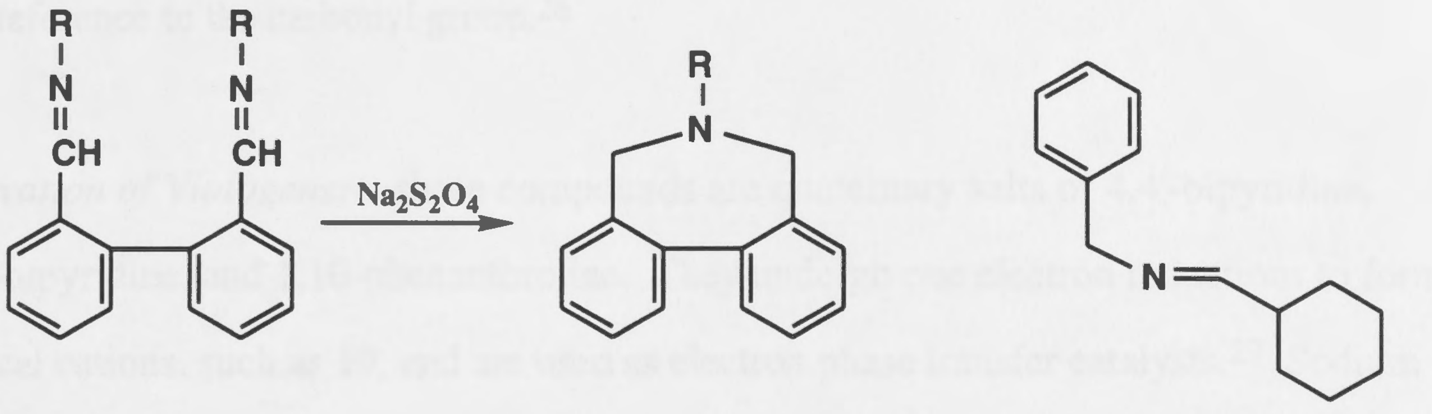

**1** **2**

**a**: $R^1 = H, R^2 = -CONH_2$ **a**: $R^1 = H, R^2 = -CONH_2$

Figure 1: Reduction of pyrimidine salts, including analogues of $NAD^+$, by $S_2O_4^{2-}$.

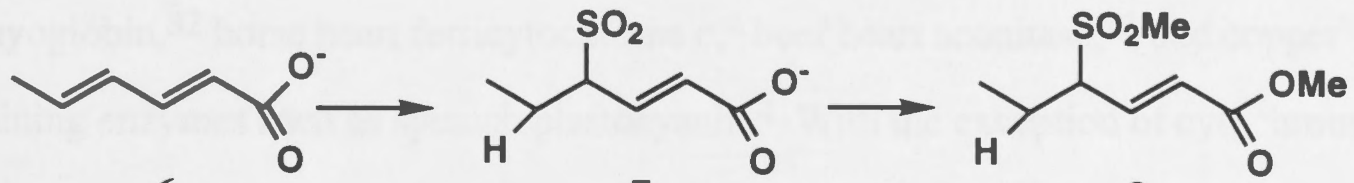
*Imines:* there have been fewer reports of sodium dithionite being used to reduce imines. However, a paper by Hawthorne *et al* described the preparation of a series of substituted dibenzazepines, **4**, by reaction of Schiff bases, **3**, with aqueous sodium dithionite.[18]

Some later work dealt with the reaction of sodium dithionite with a number of oximes and imines such as **5** in a mixed dmf/water/bicarbonate medium.[19]



**3** **4** **5**

*Alkenes and acylsulphones:* a series of alka-2,4-dienoic acids, **6**, were reduced to a variety of products by sodium dithionite.[20] The ratios of stereoisomers of the resultant alk-3-enoic acids were dependant upon the number and size of the R groups. A sulfinate adduct, **7**, was trapped by the addition of methyl iodide, providing some information about the mechanism of this reaction. Julia *et. al.* have made use of the sulfinate intermediates formed in the reduction reaction to perform stereospecific hydrogenolysis of vinylic sulfones with sodium dithionite.[5] This methodology was adopted by Babin *et.al.* in the synthesis of Z -α,β unsaturated esters,[6] and by Harris *et.al.* to synthesise a number of ketones.[21]



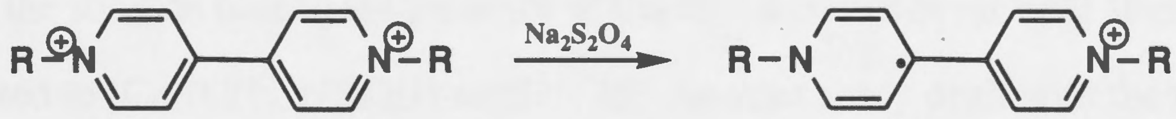
**6** **7** **8**

Figure 2: Reduction of Alka-2,4-Dienoic Acids with $S_2O_4^{2-}$

*Nitro containing compounds:* have been reduced by sodium dithionite to the corresponding amines in good yield.[22]

*Carbonyl containing compounds:* a number of recently published papers have dealt with the reduction of aldehydes and ketones to alcohols by sodium dithionite. These have included derivatives of napthacene[23] and daunomycin.[24] The yield and stereoselectivity of such reactions can be modified by the presence of agents such as β-cyclodextrin and phase transfer conditions.[25] The olefin of enones is generally reduced in preference to the carbonyl group.[26]

*Activation of Viologens:* these compounds are quaternary salts of 4,4'-bipyridine, 2,2'-bipyridine, and 1,10-phenanthroline. They undergo one electron reductions to form radical cations, such as **10**, and are used as electron phase transfer catalysts.[27] Sodium dithionite has been used to generate the radical cation which then reduces the substrate present in the organic phase. The substrates have included nitroarenes[28], α-nitro sulfones[29] and derivatives of nucleosides.[30]



**9** **10**

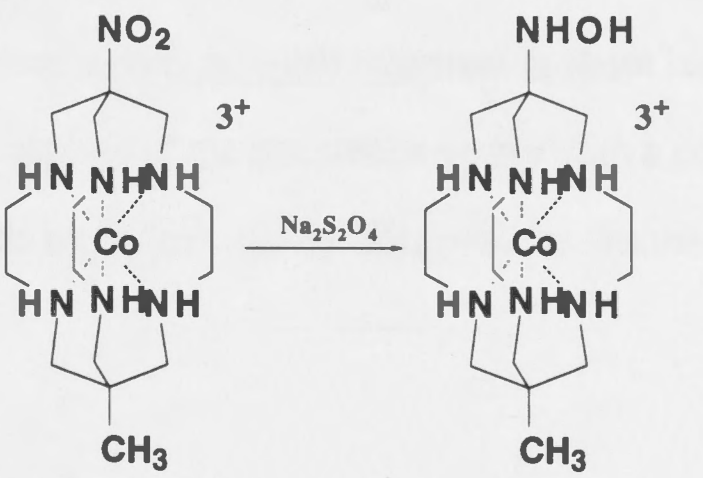
***Reduction of metal ions by $S_2O_4^{2-}$***

*Metalloenzymes:* Sodium dithionite has been used to study the kinetics and mechanism of the reduction of many electron transfer proteins. These have included iron containing enzymes such as spinach ferredoxin,[4] horse heart-[4,31] and sperm whale- skeletal muscle-metmyoglobin,[32] horse heart ferricytochrome c,[4] beef heart aconitase,[11] and copper containing enzymes such as spinach plastocyanin.[4] With the exception of cytochrome c and plastocyanin, the kinetic data obtained in these studies (under pseudo first order conditions) showed rate dependencies of the form $k_{obs} = a[S_2O_4^{2-}]^{1/2}$,[11] implying that the radical $SO_2^{\cdot-}$, not $S_2O_4^{2-}$, reduced the metal centre(s) in a one electron process. Cytochrome c and plastocyanin demonstrated behaviour synonymous with reaction with both $SO_2^{\cdot-}$ and $S_2O_4^{2-}$.[4]

*Metal Complexes:* There have been fewer reported studies of reactions of sodium dithionite with metal ions and metal complexes. These include a number of papers involving complexes of Co(III)[7,33] and some examples dealing with Fe(III)[33,34], Mn(III)[33], Mo(VI)[35] and W(VI)[35] complexes and Ni(II) ions.[36] Kinetic studies of these complexes have demonstrated that reduction of the metal centre may occur by either $SO_2^{\cdot -}$ or $S_2O_4^{2-}$ and that in the majority of cases it is the $SO_2^{\cdot -}$ radical which is the reductant.[7,33-35]

***Reduction of organic compounds coordinated to metal ions***

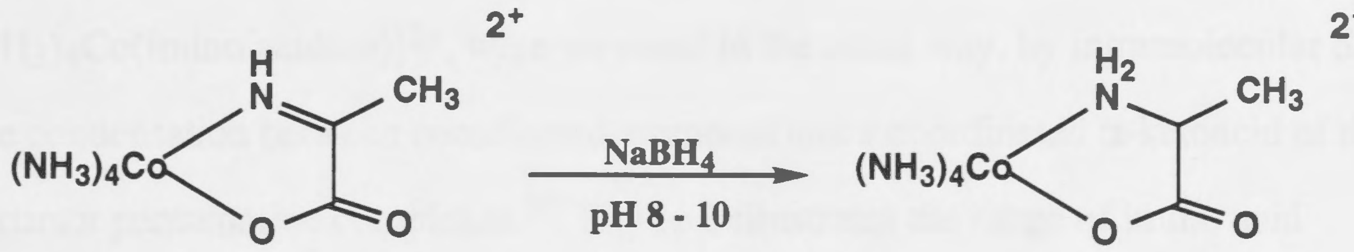
Relatively few publications have dealt with situations in which sodium dithionite may reduce the metal ion or the ligand of a complex. Those that do include studies of the reduction of cage complexes such as $[Co((CH_3, NO_2)\text{-sar})]^{3+}$, **11**.[7(b)] In this instance the nitro group is reduced much more quickly than the Co(III) and loss of the yellow colour of the solution (due to the presence of Co(III)) does not occur until after the cage is converted to $[Co((CH_3, NHOH)\text{-sar})]^{3+}$, **12**. Another study dealt with the reduction of Mn(III) porphyrins by dithionite ion. In this instance it was proposed that the reduction of Mn(III) to Mn(II) took place following the generation of a radical anion by attack of dithionite on the periphery of the porphyrin ring.[9]




**11** **12**

***THIS STUDY***

The earliest report of an α-imino acid complex of Co(III) noted that it was possible to reduce the imine complex (**14**) with sodium borohydride, to form the corresponding alaninato complex, **15**, without much reduction of the metal centre.[37] Further work examined the reduction of racemic mixtures of α-imino acid complexes of the form $[(en)_2Co(NHC(R)COO)]^{2+}$ ($R = CH_3, CH(CH_3)_2$) by borohydride. Analysis of the diastereoisomers that resulted from the reduction reaction revealed a ΛR, ΔS : ΛS, ΔR ratio of 6 : 4 for both alaninato and valinato complexes, compared to the equilibrium diastereoisomeric ratios ΛR, ΔS : ΛS, ΔR of 6 : 4 for the valinato and 1 : 1 for the alinato complex.[38]



14 15

The purpose of the study described in the following pages was to investigate the reduction, by sodium dithionite, of α-imino acids coordinated to Co(III). In doing so there were a number of important factors to consider. In particular, it was necessary to establish the conditions under which the imino acid and not the metal centre was reduced, the range of imino acids that could be reduced, and the stereoselectivity of the reduction reaction. An understanding of the mechanism of the reduction reaction was also salient, initiating some investigations which provided information about intermediates and the reaction pathway. These aspects of the reaction, together with a comparison of the reduction of α-imino acids by $S_2O_4^{2-}$ and by $BH_4^-$ will be discussed.

## Results and Discussion

### *SYNTHESES*

Complexes of the form $[(en)_2Co(amino\ acidato)]^{2+}$ were prepared using methods similar to those described by Chong *et. al.*[40] The complex $[(en)_2Co(OH_2)(OH)](ClO_4)_2$ and the amino acid were dissolved in dmso and the mixture heated to 80 °C for 45 to 60 minutes. The product amino acidato complex was isolated from the reaction mixture by ion exchange chromatography and recrystallised from dilute hydrochloric acid. In some instances, most notably in the preparation of [$(en)_2Co$(phenyl-gly)], the use of [$(en)_2Co(dmso)_2]^{3+}$ improved the yield of the desired complex.[41] With the exception of [$(en)_2Co(gly)]^{2+}$, the amino acidato complexes were oxidised by thionyl chloride to form the corresponding α-imino acidato complexes.[39] The complex [$(en)_2Co$(gly-im)]$^{2+}$ was prepared by oxidising the amino acid with $PBr_3$.[42] Tetraammine complexes, [$(NH_3)_4Co$(imino acidato)]$^{2+}$, were prepared in the usual way, by intramolecular Schiff base condensation between coordinated ammonia and a coordinated α-ketoacid of the precursor pentammine complexes.[37] Figure 3 illustrates the range of imino acid complexes utilised in this study.

***Synthesis of (p)-[trenCo(ala)]$^{2+}$.***

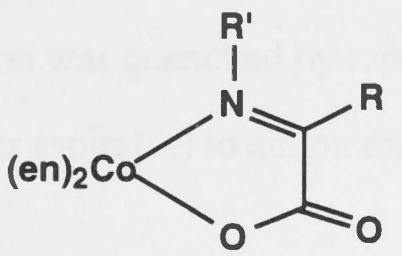
This complex was prepared in a simple, 'one pot' synthesis. Hydrogen peroxide was added, in small portions, to a slurry of tren.3HCl, Co(II), L-alanine, NaOH and activated charcoal in water. After the mixture had been stirred vigorously for a further 15 - 20 minutes it was filtered and the product, (p)-[trenCo(ala)]$^{2+}$, isolated from the filtrate by ion-exchange chromatography and recrystallised from dilute HCl. The yield, 70%, compares very favourably with the published, multi-step, syntheses of similar [trenCo(amino acidato)]$^{2+}$ complexes.[40]

Some attempts were made to synthesise [$(en)_2Co(ala)]^{2+}$ by the above method. However, the desired complex was obtained in relatively low yields (around 30%), and complexes such as [$(en)_3Co]^{3+}$ and [$(en)_2Co(ala)_2]^{+}$ were significant by-products.

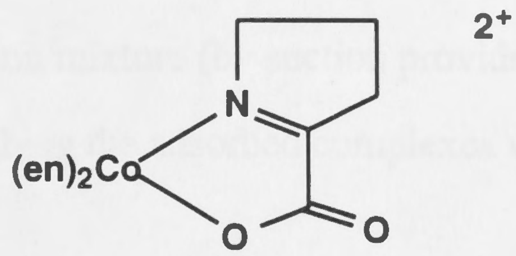
## *REDUCTION OF [$N_4Co(NHC(R)COO)]^{2+}$ BY DITHIONITE*

### *A General Description of the Reaction.*

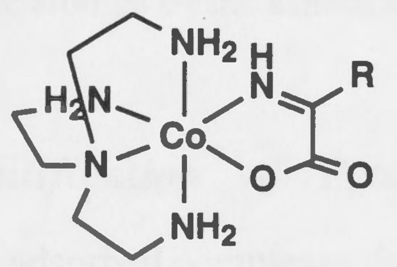
Sodium dithionite was added to a buffered solution of a [$N_4Co$(α-imino acidato)]$^{2+}$ complex and the resulting solution stirred at 25 °C for up to half an hour. During the reaction the solution's colour changed slightly, from yellow orange to red orange. The odour of $SO_2$ was apparent and a faint suspension, most probably colloidal sulphur,



**16** R' = H, R = H
**17** " R = $CH_3$
**18** " R = $CH_2CH_3$
**19** " R = $CH(CH_3)_2$
**20** " R = $(CH_2)_2SCH_3$
**21** " R = $CH_2(4\text{-}C_6H_4OH)$
**22** " R = $(CH_2)_4NH_2$
**23** " R = $C_6H_5$
**24** " R = CH=$CH_2$ **(reduction of olefin)**
**25** " R = CH=CH(3-$C_6H_4$OH) **(mixture of products)**
**26** R' = $CH_3$, R = $CH(CH_3)_2$ **(no reduction)**

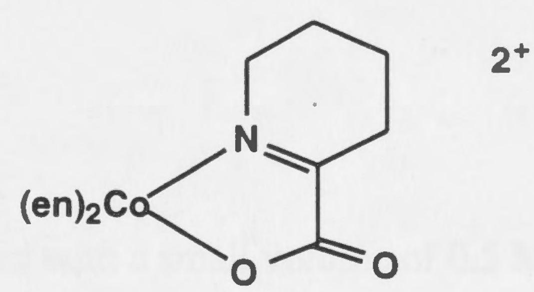


**30** **(no reduction)**

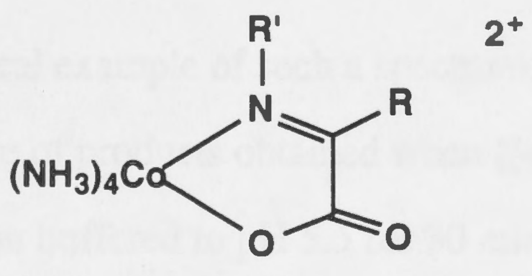


**17c**

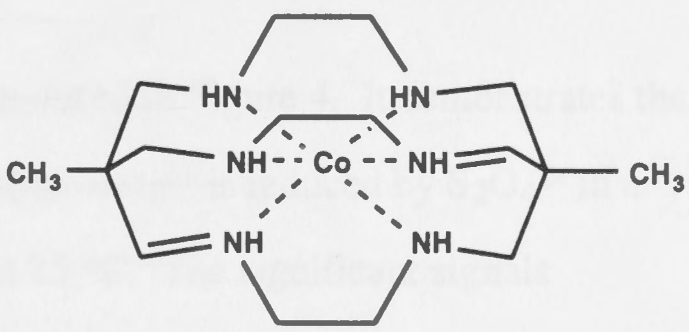
**17a** $N_4$ = $[(NH_3)_4Co(ala\text{-}im)]^{2+}$
**17b** $N_4$ = $[(en)_2Co(ala\text{-}im)]^{2+}$
**17c** $N_4$ = (p)-trenCo(ala-im)$]^{2+}$
**17d** $N_4$ = $[(bipy)_2Co(ala\text{-}im)]^{2+}$



**31** **(no reduction)**



**27** R' = H, R = $(CH_2)_2COOH$
**28** R' = H, R = $C_6H_5$
**29** R' = $CH_3$, R = $CH_3$ **(no reduction)**



**32** **(no reduction)**

Figure 3: Co(III) coordinated imines and α-imino acids used to examine the properties of sodium dithionite as a reducing agent

became noticeable about 15 minutes after the reaction had commenced. The reduction reaction was quenched by rapidly adsorbing the reaction mixture (by suction provided by a water aspirator) to an ion exchange column and washing the adsorbed complexes with water.

Earlier work[38] investigated the stereoselectivity of the reduction of the α-imino acid complexes $[(en)_2Co(ala\text{-}im)]^{2+}$ and $[(en)_2Co(val\text{-}im)]^{2+}$ by $BH_4^-$ and a number of alkyl substituted boranes. For the purposes of comparison, these complexes were used in examining the ability of dithionite to stereoselectively reduce the imino-acids. They were also used to investigate the effects of parameters such as concentration, pH and the choice of tetraamine coligand ($N_4$) on the reaction. The valine imine complex was also used in some studies of the kinetics of the reduction reaction.

***Identification of Products.***

The adsorbed complexes described above were washed with a small volume of 0.5 M HCl to ensure the removal of any Co(II) that might have formed and then the remaining material on the column was collected in a single fraction using 3 M HCl. The acid was removed by evaporation and the residual solid dissolved in $D_2O$ for $^1H$ nmr spectro~~metry~~.

A typical example of such a spectrum is reproduced in Figure 4. It demonstrates the mixture of products obtained when $[(en)_2Co(val\text{-}im)]^{2+}$ is reduced by $S_2O_4^{2-}$ in a solution buffered to pH 5.5 for 30 minutes at 25 °C. The significant signals corresponding to the amino- and imino- acidato complexes are described in Table 1. The signals in the spectrum were identified by comparison with spectra of samples of authentic $[(en)_2Co(val)]^{2+}$ and $[(en)_2Co(val\text{-}im)]^{2+}$. The signals due to the diastereoisomers of $[(en)_2Co(val\text{-}im)]^{2+}$ have been identified previously.[44]

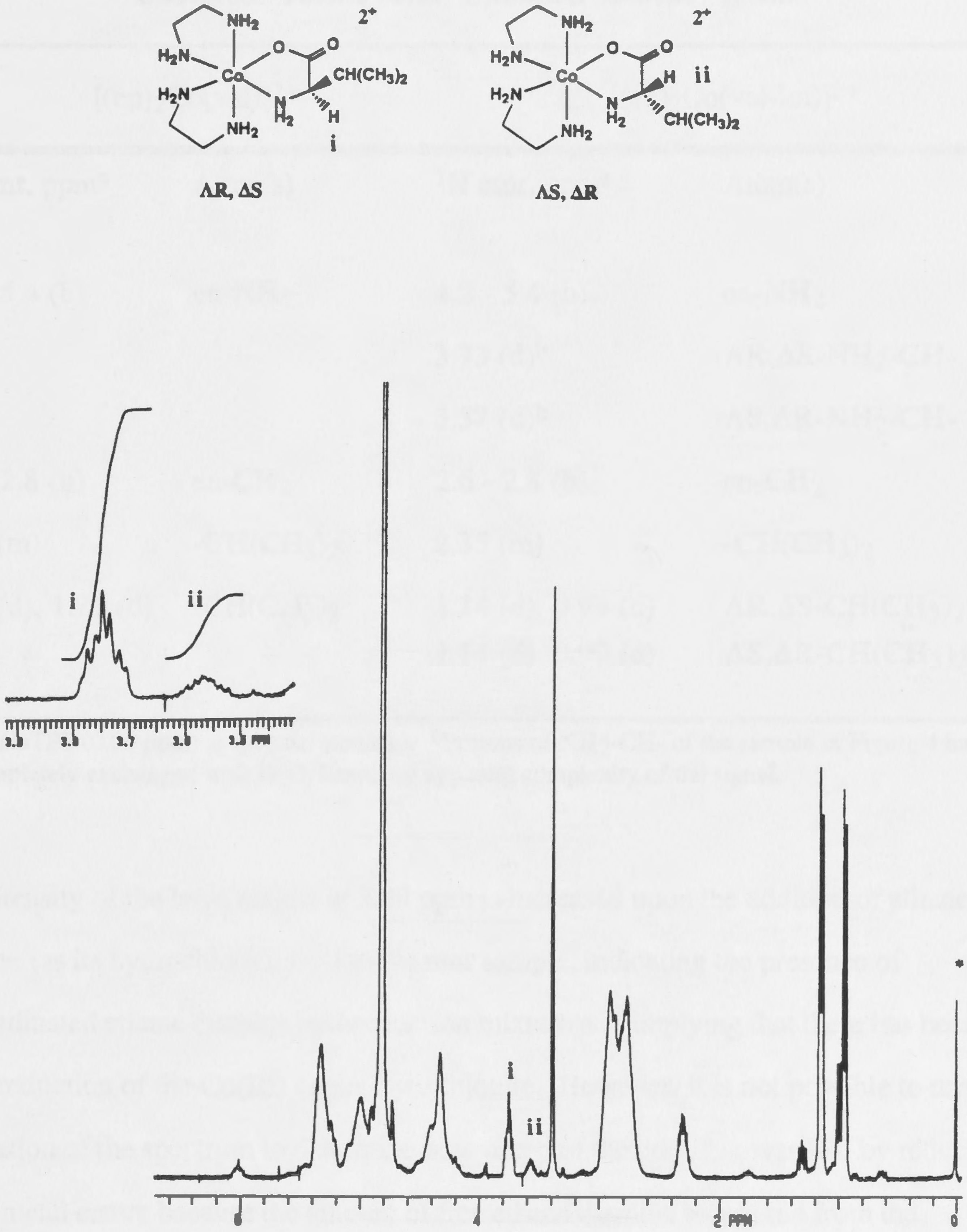



Figure 4: $^{1}$H nmr spectrum (0.1 M DCl, NaTPS as internal standard) of the mixture of complexes recovered after reacting $Na_2S_2O_4$ with $[(en)_2Co(val\text{-}im)]^{2+}$ at pH 5.5 (0.5 M Bis Tris) for 30 minutes at 25 °C. Inset: integration of signals due to the protons on the $\alpha$-carbon of the ΛR, ΔS (3.73 ppm) and ΛS, ΔR (3.57 ppm) isomers.

***Table 1: Assignment of Signals from Figure 4 to the Atoms of Chelated Valine and Chelated Valine Imine.***

| $[(en)_2Co(val)]^{2+}$ | | $[(en)_2Co(val\text{-}im)]^{2+}$ | |
|---|---|---|---|
| $^1$H nmr, ppm[a] | Atom(s) | $^1$H nmr, ppm[a] | Atom(s) |
| 4.2 - 5.4 (b) | en-N**H**$_2$ | 4.2 - 5.4 (b) | en-N**H**$_2$ |
| | | 3.73 (d)[b] | ΛR,ΔS-$NH_2$-CH- |
| | | 3.57 (d)[b] | ΛS,ΔR-$NH_2$-CH- |
| 2.6 - 2.8 (b) | en-C**H**$_2$ | 2.6 - 2.8 (b) | en-C**H**$_2$ |
| 3.14 (m) | -C**H**($CH_3$)$_2$ | 2.35 (m) | -C**H**($CH_3$)$_2$ |
| 1.32 (d), 1.29 (d) | -CH(C**H**$_3$)$_2$ | 1.14 (d), 0.94 (d) | ΛR,ΔS-CH(C**H**$_3$)$_2$ |
| | | 1.14 (d), 0.99 (d) | ΛS,ΔR-CH(C**H**$_3$)$_2$ |

[a]$D_2O$, NaTPS (0.00 ppm) as internal standard. [b]Protons of N**H**$_2$-CH- of the sample in Figure 4 have not completely exchanged with $D_2O$, hence the apparent complexity of the signal.

The intensity of the large singlet at 3.39 ppm is increased upon the addition of ethane diamine (as its hydrochloride salt) to the nmr sample, indicating the presence of uncoordinated ethane diamine in the reaction mixture and implying that there has been some reduction of the Co(III) centre by dithionite. However, it is not possible to use the integration of the spectrum to determine how much of the complex was lost by reduction of the metal centre because the amount of free ethane diamine recovered from the chromatography column was variable. Instead a comparison of the weight of the imino acidato complex that was added to the reaction mixture with the weight of the dried residues obtained from the ion exchange column after quenching the reaction indicates that there has been a loss of between 5 and 10% of the original weight. The molecular weights of the two species are virtually the same so it can be argued that around 5 to 10% of the complex has been lost as a result of reduction of Co(III) to Co(II) by dithionite.

The presence of signals at 1.32 and 1.29 ppm, corresponding to the γ-methyl groups of the valine imine, indicate that not all of the imine has been reduced. The efficacy of dithionite in reducing the imine may be estimated by comparing the integral of these signals with that of the γ-methyls of the valinato complex. In this example, 94% of the complexes are present as the amino acidato (reduced) complex after a reaction time of 30 minutes . However, it is difficult to obtain more than an estimate of dithionite's reducing ability because of the difficulty in determining the relative quantities of imino- and amino-acidato complexes that have been reduced at the metal centre.

***Stereoselectivity of the Reduction Reaction***

The product, $[(en)_2Co(val)]^{2+}$, exists as a mixture of diastereoisomers: ΛR, ΔS and ΛS, ΔR. The proton on the α-carbon of each of the isomers can be assigned to a set of signals in Figure 4.[44] The two sets of signals are quite distinct and their integrals will determine the relative proportion of each of the diastereoisomers and hence the diastereoselectivity of the reduction reaction. The equilibrium ratio of these diastereoisomers has been found[44] to be ΛR, ΔS : ΛS, ΔR = 6.0 : 4.0. In addition, some previous work examined the stereoselectivity of the reduction of $[(en)_2Co(val\text{-}im)]^{2+}$ by $BH_4^-$. In this instance, the ratio of isomers in the product was also ΛR, ΔS : ΛS, ΔR = 6 : 4.[38] By comparison, the experiments described here show that reduction of $[(en)_2Co(val\text{-}im)]^{2+}$ with $S_2O_4^{2-}$ resulted in a mixture of diastereoisomers having a ratio ΛR, ΔS : ΛS, ΔR of 4.2 : 1.0;[45] reduction of the valine imine ligand by $S_2O_4^{2-}$ has occurred with significantly greater stereoselectively than the corresponding reaction involving $BH_4^-$.

***Variation of Reaction Parameters.***

*Change of Reaction Conditions*

A number of the parameters defining the reaction conditions were varied in order to optimise the yield and the stereoselectivity of the reduction reaction. These included the concentration of the substrates, pH of the solution, temperature of the reaction mixture, and the presence or absence of $O_2$.

In general, the reduction was performed with a molar ratio of dithionite : complex of 5 : 1, the concentrations of $Na_2S_2O_4$ and $[(en)_2Co(val\text{-}im)]^{2+}$ being 0.10 M and 0.02 M respectively. If the concentrations of the species (particularly the dithionite) were raised above these levels a fine, very insoluble, pale orange suspension formed. $^1$H nmr spectra (in 6 M DCl) of this material revealed it to be a mixture of amino and imino acidato complexes. Elemental microanalysis implied that the counter ions were a complex mixture of dithionite and its decomposition products. Dithionite reacts readily with $H_2O$[46] and $O_2$:[47]

$$2S_2O_4^{2-} + H_2O = S_2O_3^{2-} + 2HSO_3^- \qquad \textbf{IV}$$

$$S_2O_4^{2-} + O_2 = S_2O_6^{2-} \qquad \textbf{V}$$

If the dithionite : complex ratio was decreased to 3 : 1 or less then reduction of the imine was incomplete because of the loss of dithionite ion to competing reactions. On the other hand, if the ratio was increased beyond 5 : 1 reduction of $Co^{3+}$ became a significant problem. Under these circumstances very little $[(en)_2Co(val)]^{2+}$ was recovered after quenching the reaction; instead a black precipitate, presumably CoS or Co,[7] formed during the reaction and quantities of $Co^{2+}$ were isolated from the quenched reaction mixture by ion exchange chromatography.

When oxygen was removed from the reaction mixture by flushing the solution containing the complex with nitrogen before adding sodium dithionite, the reaction time was reduced from thirty to five minutes, presumably because dithionite was no longer reacting with $O_2$ and no longer being diverted from reaction with $[(en)_2Co(val\text{-}im)]^{2+}$.

Reducing the temperature of the reaction mixture from 25 to 0 °C reduced the rate of the reaction but did not affect the stereoselectivity of the reaction.

The pH of the solution was found to have a dramatic effect on the reduction reaction. $Na_2S_2O_4$ and $[(en)_2Co(val\text{-}im)]^{2+}$ were mixed in buffers having a pH in the range 3.5 to 9.5, inclusively. The mixture of products were isolated by ion exchange chromatography and identified by $^1H$ nmr spectrometry. Table 2 details the results of these experiments. At a pH of more than 5.5 there was more reduction of the metal centre and a corresponding decrease in the amount of complexes recovered. In addition, there was a decrease in the proportion of amino to imino acid complexes that were recovered after quenching the reaction. At a pH of 5.5 or less there was less reduction at the metal centre and more amino acid complex was recovered.

***Table 2: Influence of pH on the Outcome of the Reduction of $[(en)_2Co(val\text{-}im)]^{2+}$ by $S_2O_4^{2-}$.***

| pH | Buffer[a] | Recovered complexes[b], % | Reduction of imino acid[c], % |
|---|---|---|---|
| 3.0 | Acetate | 90 | 97 |
| 4.1 | Acetate | 85 | 98 |
| 5.5 | Bis - Tris | 70 | 94 |
| 6.5 | Bis - Tris | 50 | 87 |
| 7.5 | Bis - Tris | 35 | 25 |
| 8.5 | HEPES | 30 | 20 |
| 9.5 | Carbonate | 10 | 18 |

[a][Buffer] = 0.5 M. [b]Percentage of recovered complexes to the nearest 5%. Estimated error in percentage of recovered complexes: 15%. [c]Reduction of imino acid as a percentage of total (amino- + imino-acidato) complexes isolated. Estimated error in percentage reduction of imino acid complexes: 5%.

The amount of reduced (amino acid) complex recovered from the reaction mixture decreased with increasing pH. Explaining why this is so is not easy.

Firstly, the change in product distribution (of imino and amino acid complexes) with pH may be due to hydrolysis of dithionite ion under acid conditions.[46] Under more basic conditions there is more dithionite ion and hence more reducing agent available to take part in the reactions under investigation. For this reason, previous studies (including those involving the reduction of the metal centre of various complexes) have been performed in the pH range 6.3 to 13.0.[8,9]

The present study involved two reduction reactions; that of the metal centre and that of the imino acid ligand. The rate of reduction of Co(III) increased with an increase in pH (as evidenced by the decrease in the amount of complexes recovered after the reaction,Table 2) and this can be said to be due to the higher concentration of dithionite ion at higher pH. The proportion of reduced (amino acid) complexes in the recovered material decreased with an increase in pH. This seems to imply that the rate of reduction of the imino acid complex is pH dependant and decreases with an increase in pH, behaviour completely opposite to that of the metal centre. Reduction of the metal centre hampered attempts to determine the rate of reduction of $[(en)_2Co(val\text{-}im)]^{2+}$ by $^1H$ nmr spectrometry or uv-vis spectroscopy so it was not possible to confirm this hypothesis.

In summary, conditions which result in reduction of the imine group of the complex $[(en)_2Co(val\text{-}im)]^{2+}$ are: aqueous solution of pH 3.0 to 4.5 and an excess (5 times molar excess) of dithionite ion over the complex at 25 $^oC$. The reduction is complete within thirty minutes and the stereoselectivity of the reaction is such that ΛR, ΔS : ΛS, ΔR of the product valinato complex was 4.2 : 1.0. By contrast, the same reduction may be carried out with only a 1 : 1 molar ratio of $[(en)_2Co(val\text{-}im)]^{2+}$ and $BH_4^-$ in mildly alkaline conditions at 25 $^oC$ but the isomer distribution of the amino acidato complexes shows rather less stereoselectivity: ΛR, ΔS : ΛS, ΔR = 6 : 4.

*Change of α-Imino Acid*

The facility with which dithionite ion reduces different imino acidato complexes was studied using the complexes depicted in Figure 3. These include a range of α-imino derivatives of naturally occurring amino acids as well as synthetic imino acidato complexes. The reduction reactions were carried out under the same conditions as those described previously for reductions of $[(en)_2Co(val\text{-}im)]^{2+}$.

In general, imines of the form HN=C (**16** - **23**, **27**, **28**) were reduced by $S_2O_4^{2-}$ and those of the form RN=C (**26**, **29**, **30**, and **31**) were not. This is in direct contrast to the capability of $BH_4^-$, which can reduce the imine of species such as $[(NH_3)_4Co(pro\text{-}im)]^{2+}$.[48] $^1H$ nmr spectra of the residues recovered after reaction of $S_2O_4^{2-}$ with complexes of **26**, **29**, **30**, and **31** contained no signals due to the corresponding amino acidato complexes. Instead they contained large signals due to the presence of uncoordinated ethane diamine, indicating that reduction of the metal centre and consequent loss of the complex had occurred. Changing the reaction conditions, such as reducing the number of molar equivalents of $S_2O_4^{2-}$ used in the experiment, or removing $O_2$ from the solution did not yield any more of the amino acidato complexes. Under these circumstances it was difficult to determine whether the imine could *not* be reduced under the experimental conditions or whether it *was* reduced, followed by subsequent, rapid, reduction of the Co(III) centre of the resulting amino acid complex. In order to gain more information the 'cage' complex **32**, which contains the group RN=C and in which the Co(III) is much more stable towards reduction, was mixed with dithionite and monitored by $^{13}C$ nmr spectrophotometry. No signals corresponding to the reduced imine were detected in the resulting spectra. This result tends to imply that the RN=C imino acidato complexes are not reduced to the corresponding amino acidato complexes before the cobalt centre is reduced and could reflect the increased electronegativity of the imine upon alkylation.

The $[(en)_2Co(gly\text{-}im)]^{2+}$ complex, **16**, proved to be very susceptible to reduction of Co(III). Under the usual reaction conditions the solution turned from clear orange to brown with a black suspension within seconds of addition of $S_2O_4^{2-}$. However, if a 1 : 1 ratio of $S_2O_4^{2-}$ : $[(en)_2Co(gly\text{-}im)]^{2+}$ was mixed in a deoxygenated buffer solution at pH 4.1 for 30 seconds then $[(en)_2Co(gly)]^{2+}$ was isolated from the quenched reaction mixture virtually quantitatively. $[(en)_2Co(gly\text{-}im)]^{2+}$ was also reduced by $BH_4^-$, this time using the usual reaction conditions for this reducing agent (slightly basic solution, 1 : 1 ratio of $BH_4^-$ and complex, 30 seconds reaction time).

The diastereoselectivities of those reduction reactions involving complexes of bis- ethane diamine were determined in a similar manner to that described for isomers of $[(en)_2Co(val\text{-}im)]^{2+}$. Regardless of the nature of the side chain on the imino acid, the isomer distribution of the resulting amino acids was approximately ΛR, ΔS : ΛS, ΔR = 6 : 4 This is the same degree of stereoselectivity exhibited by reductions of $[(en)_2Co(ala\text{-}im)]^{2+}$ by $BH_4^-$ and a number of alkyl substituted borane reagents.[38]

In order to evaluate an aspect of the regioselectivity of the reduction reaction, dithionite was mixed with **24** and **25**. Borohydride has been found to reduce the alkene of **24**, in preference to the imine and dithionite proved to behave in the same manner. Reduction of **25** by dithionite yielded a complex mixture of products that proved difficult to separate. $^1H$ nmr spectra of the fractions implied that species in which either or both of the alkene and imine had been hydrogenated, but it was difficult to determine the relative quantities of each species.

*Change of Coligand(s)*

A group of complexes, $[N_4Co(ala\text{-}im)]^{2+}$ ($N_4 = (NH_3)_4$, $(en)_2$, tren, $(bipy)_2$) were used to gain a measure of the utility of dithionite as a reducing agent for different imino acid complexes. The ease of generation of Co(II) in these reactions has the order: $(bipy)_2 \gg (NH_3)_4 > (en)_2 =$ tren. Dithionite ion is not a useful reagent for reducing the imine of

$[(bipy)_2Co(\text{imino acidato})]^{2+}$ complexes, the complex is not stable enough to reduction of the metal ion. Some Co(II) production occurs during reduction of $[(NH_3)_4Co(\text{ala-im})]^{2+}$, and even less occurs during the reduction of $[(en)_2Co(\text{ala-im})]^{2+}$ and $[trenCo(\text{ala-im})]^{2+}$. A comparison between the reduction of $[(en)_2Co(\text{ala-im})]^{2+}$ and $[(NH_3)_4Co(\text{ala-im})]^{2+}$ is given in Table 3.

Multidentate ligands are known to stabilise the Co(III) state relative to ammonia and unsaturated ligands such as 2,2' bipyridine are known to stabilise the Co(II) state more than the saturated amine ligands, so the order of stability towards dithionite of Co(III) in the complexes is not too surprising.

***Table 3: Influence of Coligand on the Reduction of $[N_4Co(\text{ala-im})]^{2+}$ at pH 4.06.***

| Time, min | $(en)_2Co(\text{ala-im})]^{2+}$ | | $[(NH_3)_4Co(\text{ala-im})]^{2+}$ | |
|---|---|---|---|---|
| | Recovered Complexes[a], % | Reduced Species[b] % | Recovered Complexes[a], % | Reduced Species[b] % |
| 5 | 100 | 66 | 62 | 63 |
| 15 | 92 | 80 | 0.0 | - |

[a]Estimated error in percentage of recovered complexes: 5%. [b]Reduction of imino acid as a percentage of total (amino- + imino- acidato) complexes present in the sample. Estimated error in percentage reduction of imino acid complexes: 5%.

## *KINETICS STUDIES.*

### *Variation of reaction conditions*

Variation of the conditions in which the reduction experiments were performed provided some information about the relative rates of the reactions that occurred. The change in the product distribution with change in pH of the reaction mixture (described above) is particularly noteworthy. In order to obtain more information about the reduction reaction, including the identity of the reducing species ($S_2O_4^{2-}$ and/or $SO_2^{\cdot -}$), the kinetics of the reaction were investigated by $^1H$ nmr spectrometry and by uv-vis spectroscopy.

### *$^1H$ nmr spectrometric experiments*

The reduction of $[(en)_2Co(val\text{-}im)]^{2+}$ at pD 4 was monitored by $^1H$ nmr spectrometry (Figure 5). Reduction of Co(III), evident in the broadening of the signals with time, occurs concurrently with reduction of the imine and not toward the end of the reaction, with the remaining dithionite. A further indication of Co(III) reduction is the signal at 3.39 ppm, which is due to the presence of uncoordinated ethane diamine and which increases with time. The signals of the γ-methyl groups of the imine (1.29 - 1.32 ppm) decrease with time, whilst those of the corresponding amino acid (1.14 - 0.94 ppm) increase. Reduction of the imino acid was complete within 35 minutes.

Attempts to perform similar experiments at higher pD were not successful because of the quantity of Co(II) generated.

### *uv-vis spectroscopic experiments*

An investigation of the reduction by uv-vis spectroscopy was attempted. This involved reacting (p)-$[trenCo(ala\text{-}im)]^{2+}$ with $Na_2S_2O_4$ at pH 3.9 and 5.0 under pseudo first order conditions at 20.0 °C in the absence of $O_2$. The p isomer of the tren complex (in which the oxygen of the amino acid is *trans* to the primary amine of the tran ligand) was used to avoid complicating the analysis of the spectral data with considerations of isomer distributions. The buffer solutions (acetate) were flushed with nitrogen for a minimum of twenty minutes before being used to make solutions of the complex and of dithionite. New solutions of $Na_2S_2O_4$ were prepared for each experiment to limit hydrolysis. The change in absorbance of the solution over time was monitored at 330 or 350 nm, wavelenths at which the spectra of (p)-$[trenCo(ala\text{-}im)]^{2+}$ and (p)-$[trenCo(ala)]^{2+}$ were significantly different. Various problems were encountered with these experiments. Dithionite ion was present in large excess compared to the complex and competing

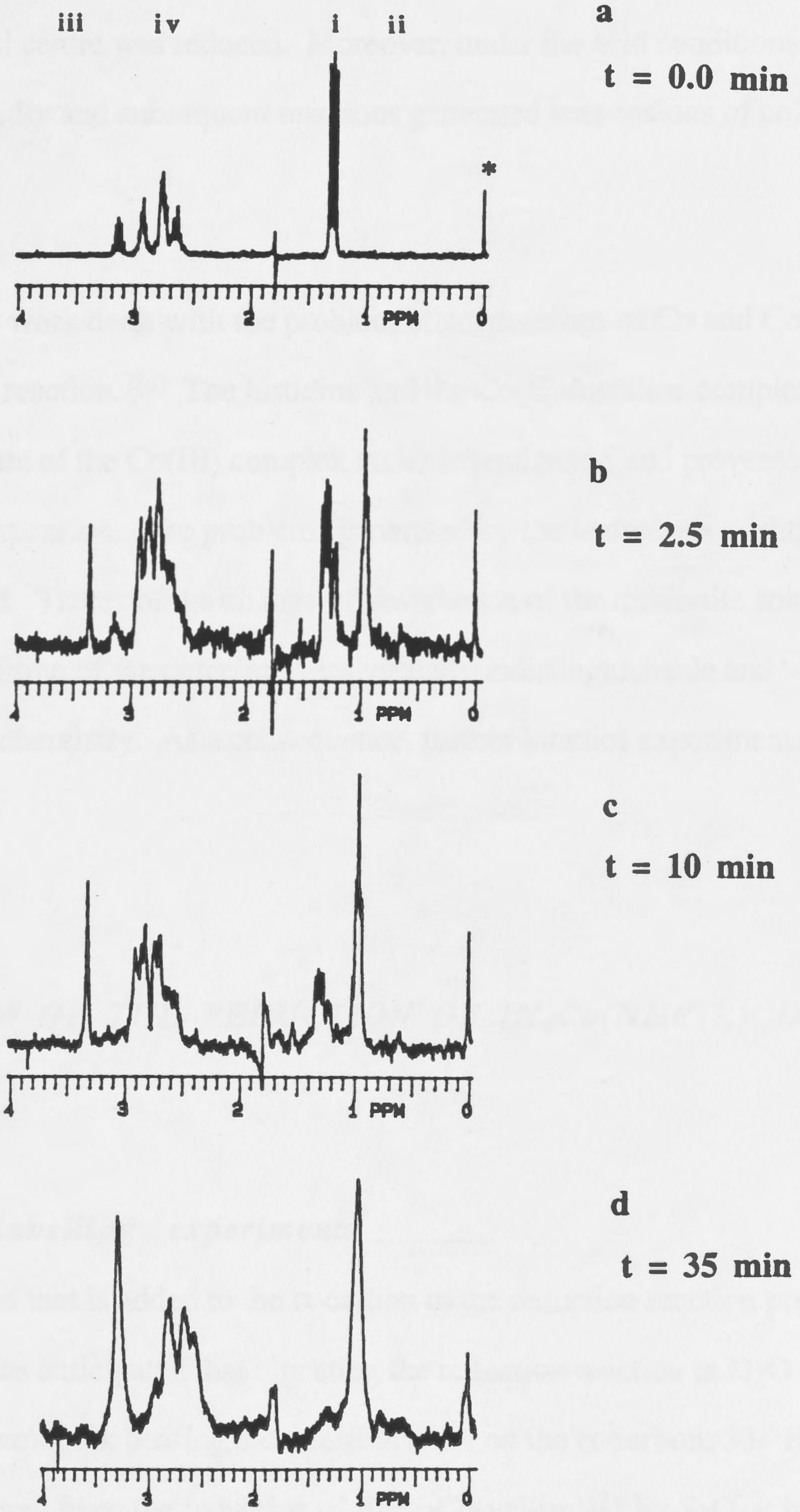



Figure 5: Reduction of $[(en)_2Co(val\text{-}imine)]^{2+}$ at pD 4 ($D_2O$) over 35 minutes.

**i**: 2 x $CH_3$ (imino acid complex), **ii**: 2 x $CH_3$ (amino acid complex), **iii**: free ethane diamine, **iv**: coordinated ethane diamine, *****: NaTPS (internal standard).

reactions for this species tended to overwhelm absorbance changes due solely to the reduction of the imine. Black suspensions, apparently of Co and/or CoS formed in the cell as the metal centre was reduced. Moreover, under the acid conditions dithionite hydrolysed rapidly and subsequent reactions generated suspensions of colloidal sulphur.[46]

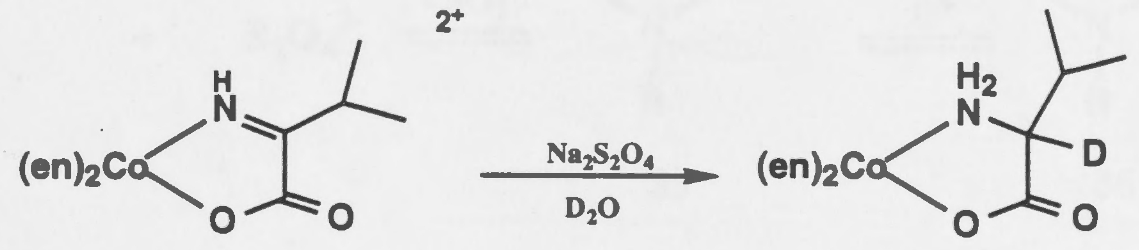
Some previous work dealt with the problem of suspensions of Co and CoS by adding histidine to the reaction.[7(a)] The histidine and the Co(II)-histidine complex did not affect the reduction rate of the Co(III) complex under investigation and prevented the formation of the black suspension. The problems generated by the hydrolysis of dithionite are not so easily solved. Traces of the change of absorbance of the dithionite solution with and without the addition of the complex were virtually indistinguishable and were dominated by the sulphur chemistry. As a consequence, further kinetics experiments were suspended.

## *MECHANISM OF THE REDUCTION OF $[N_4Co(NHC(R)COO)]^{2+}$ BY $S_2O_4^{2-}$*

### *Deuterium labelling experiments*

Since the proton that is added to the α-carbon in the reduction reaction presumably comes from $H_2O$, it was anticipated that repeating the reduction reaction in $D_2O$ would result in an amino acid complex bearing a deuterium label on the α-carbon, **33**. However, the material recovered from the reduction of $[(en)_2Co(val\text{-}im)]^{2+}$ by $S_2O_4^{2-}$ in $D_2O$ contained no labelled amino acid. The same result occurred in reductions of $[(en)_2Co(ala\text{-}im)]^{2+}$ by $S_2O_4^{2-}$ in $D_2O$. A previous study demonstrated that when $[(en)_2Co(ala\text{-}im)]^{2+}$ was reduced by $BD_4^-$ a deuterium was added to the α-carbon of the resulting amino acid.[38] This species was isolated in a similar manner to that described here for the isolation of complexes produced by the reduction of $S_2O_4^{2-}$. Thus, the label could not have been lost as the result of exchange with $H^+$ on the ion exchange column.

In a new experiment, $[(en)_2Co(val\text{-}im)]^{2+}$ was reduced by $S_2O_4^{2-}$ in $D_2O$ and the resulting complexes were isolated by precipitating them from solution by adding excess ethanol. The pale orange solid was dissolved in a minimum quantity of 6 M DCl, stirred for 10 minutes, and then diluted with $H_2O$ and adsorbed to an ion exchange column in the usual way. This time $^1H$ nmr spectrometry of the fraction containing all the complexes revealed the deuteriated valinato complex, Figure 6 (compare with Figure 4). The signals attributed to the protons of the α-methine (3.73, 3.57 ppm) are not evident in this spectrum. The same technique was used to isolate the corresponding deuteriated alaninato complex.

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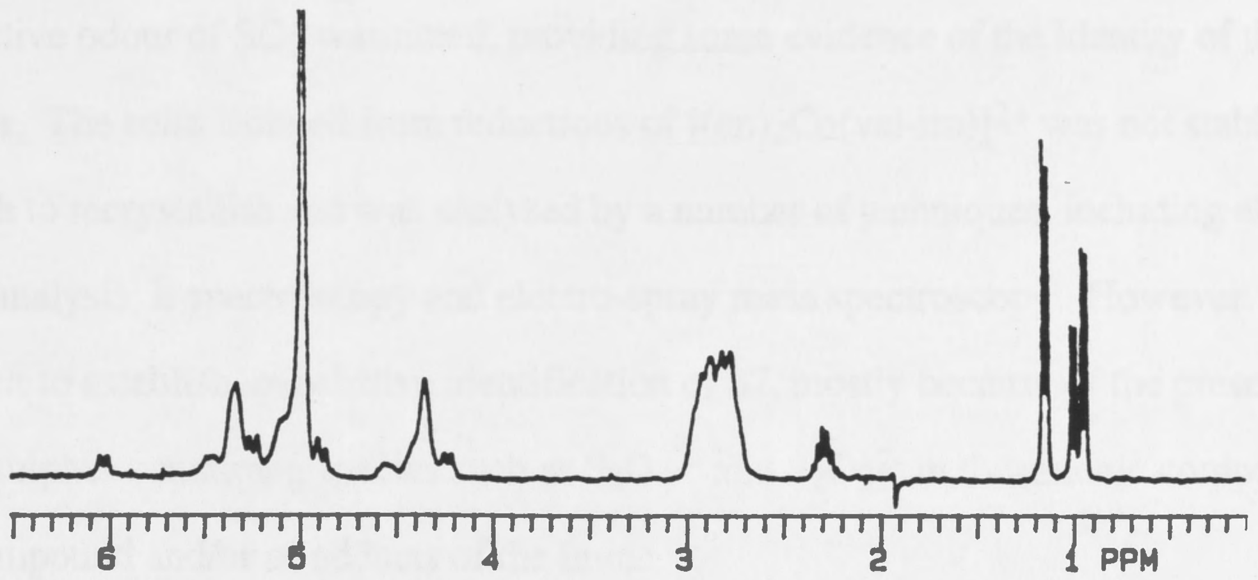
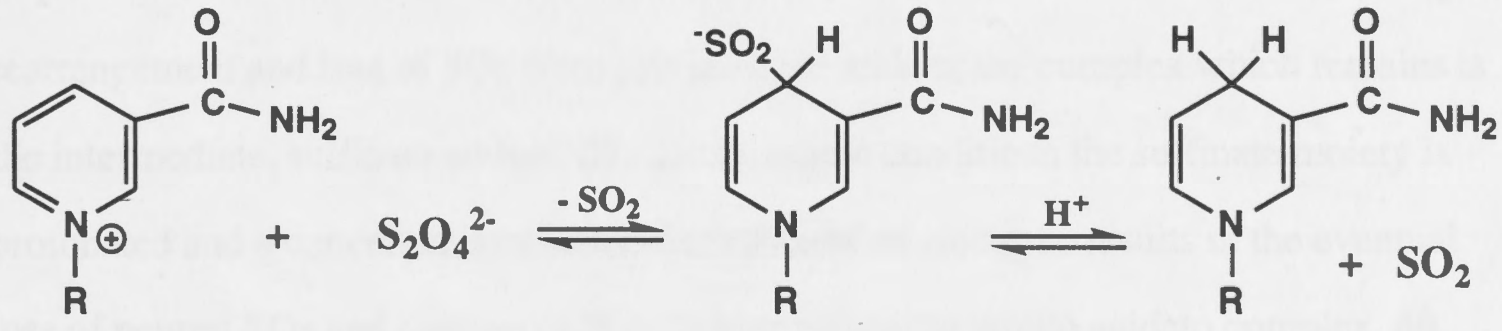



Figure 6: $^1H$ nmr spectrum (0.1 M DCl, NaTPS as internal standard) of the deuteriated α-amino acidato complex $[(en)_2Co(NH_2CD(CH(CH_3)_2)COO)]^{2+}$, **33**.

***Detection of an intermediate***

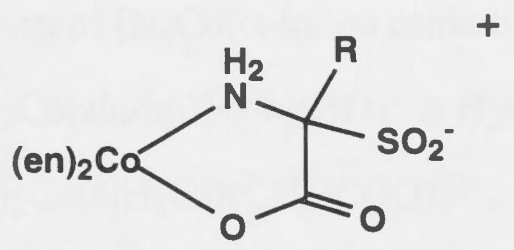
The 'loss' of a label in the above experiments implies the presence of an acid sensitive, sulphur containing intermediate which forms during the reduction process and which is destroyed during chromatographic isolation of the products of the reduction, or upon dissolving the precipitated products in acid solution. A few studies of reductions by dithionite ion have reported isolating sulfinate adducts of some organic compounds.[5,16,24(a)] For example, nicotinamides form sulfinate adducts, which are stable at high pH but which convert to dihydronicotinamides in acid conditions, Figure 7.[16] By analogy, the acid sensitive intermediates isolated in these experiments would have the form of **37**.



**34** **35** **36**

Figure 7: Sulfinate Intermediate in Reductions of Nicotinamide Derivatives.[16]

When the precipitated solids containing the intermediates were dissolved in 6 M HCl the distinctive odour of $SO_2$ was noted, providing some evidence of the identity of the species. The solid isolated from reductions of $[(en)_2Co(val\text{-}im)]^{2+}$ was not stable enough to recrystallise and was analysed by a number of techniques, including elemental microanalysis, ir spectroscopy and electro-spray mass spectroscopy. However, it was difficult to establish conclusive identification of **37**, mostly because of the presence of other sulphur containing species such as $S_2O_4^{2-}$ and $S_2O_3^{2-}$ in the anionic component of the compound and/or as adducts of the imine.



37

***Mechanism of the reduction of [$N_4Co$($\alpha$-imino acidato)]$^{2+}$ by $S_2O_4^{2-}$***

It may be argued, on the basis of comparison with previous examples,[5,16,24(a)] that the identity of the intermediate isolated from the reduction reaction is **37**. It follows that the mechanism of the reduction may be described as a process whereby the nucleophilic sulphur atom of dithionite ion initially adds to the $\alpha$-carbon of the imino acid, **38**. Upon rearrangement and loss of $SO_2$ from this unstable adduct, the complex which remains is the intermediate, sulfinate adduct, **37**. Under acidic conditions the sulfinate moiety is protonated and a concerted intramolecular 'cascade' of electrons results in the eventual loss of neutral $SO_2$ and capture of H or D to generate the amino acidato complex, **40**, Figure 8.

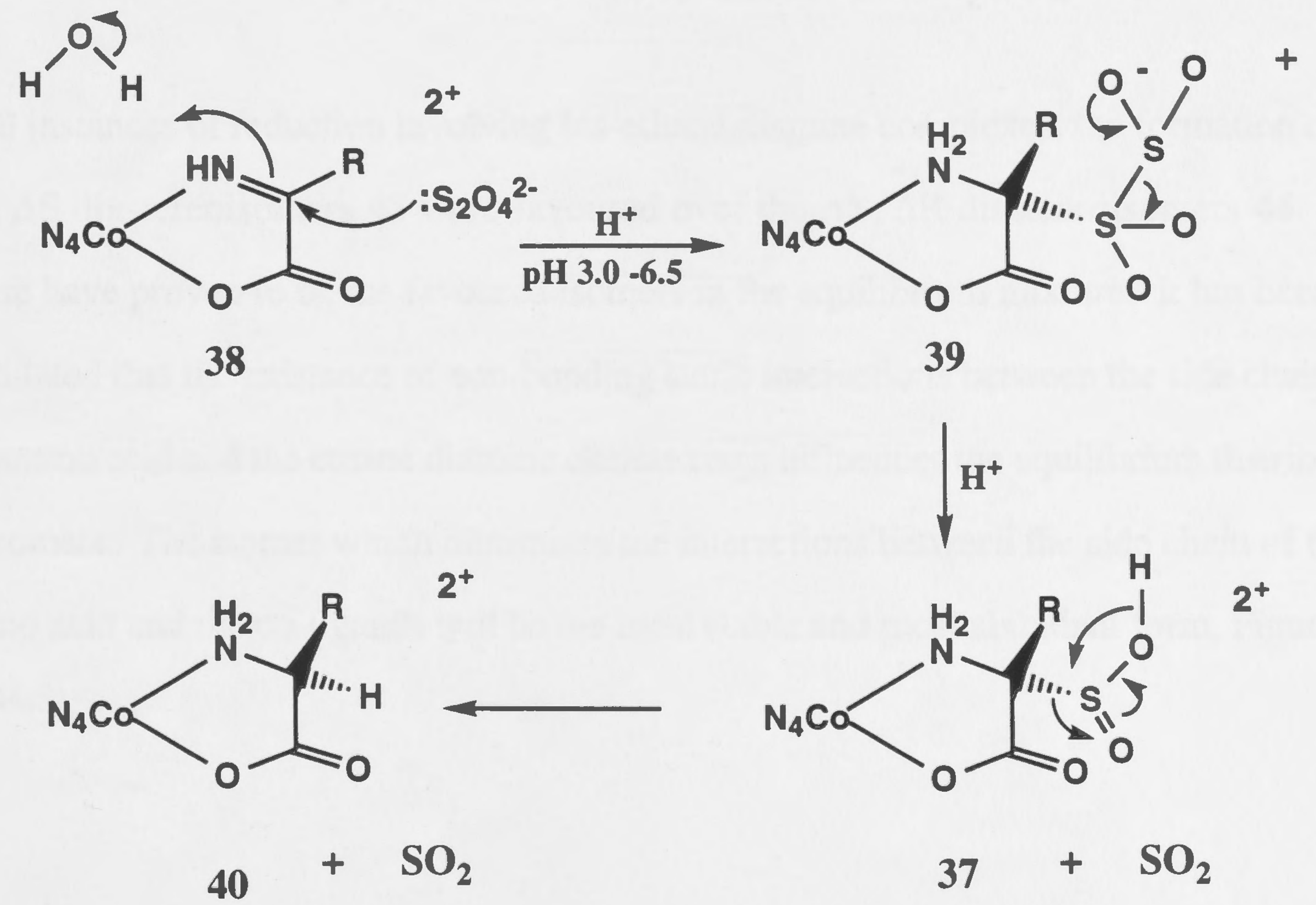


Figure 8: Reduction of $\alpha$-imino acids by $S_2O_4$.$^{2-}$

The mechanism of the reduction of $[N_4Co(\alpha\text{-imino acidato})]^{2+}$ by $BH_4^-$ is distinctly different. Reduction of $[(en)_2Co(ala\text{-}im)]^{2+}$ by $BD_4^-$ in $H_2O$ resulted in the deuteriated amino acidato complex, $[(en)_2Co(NH_2C\mathbf{D}(CH_3)COO)]^{2+}$. The implication here is that the deuterium (or protium) comes from the borohydride species itself and that there must be a transition state, **41**, in which the D-αC bond is formed as the result of a concerted intermolecular 'cascade', Figure 9. This mechanism is in general agreement with that proposed for reduction of species such as carbonyls by $BH_4^-$.[50]

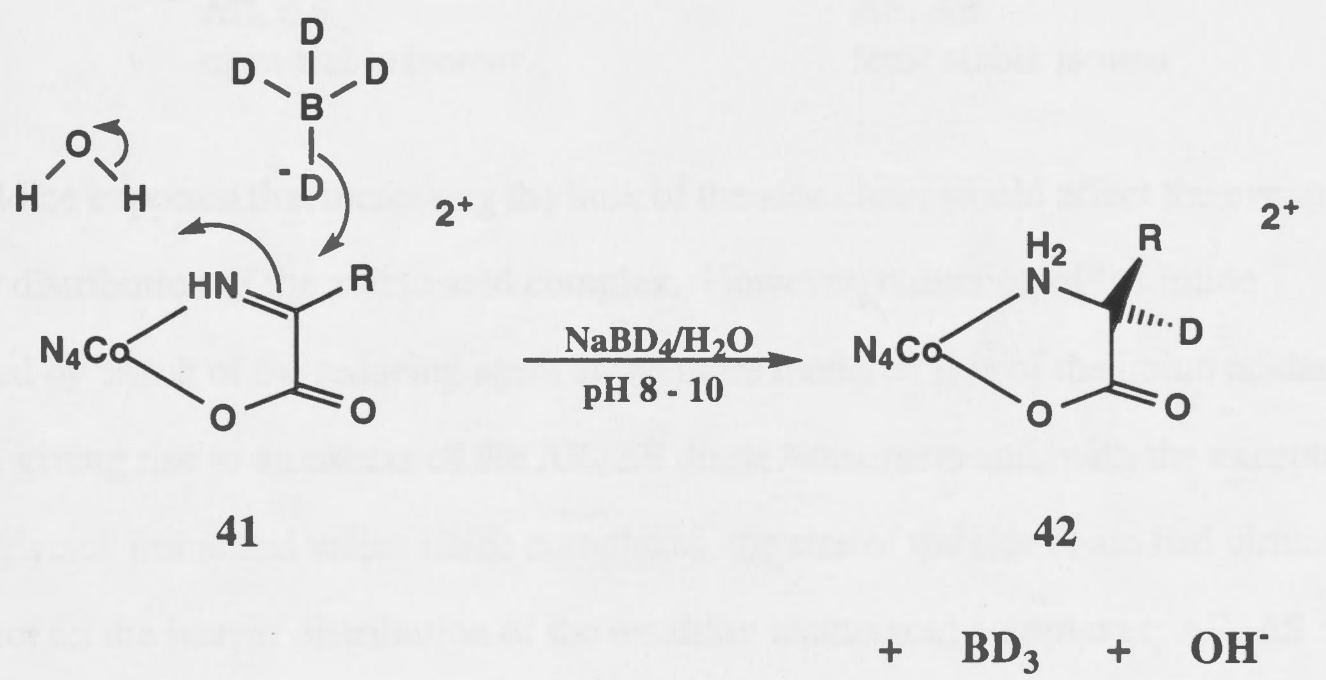
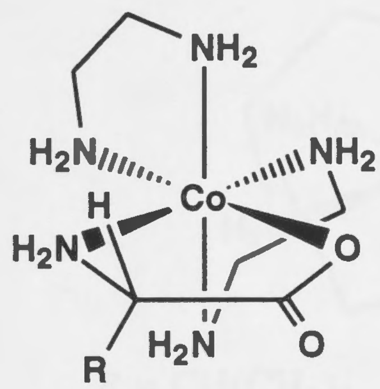
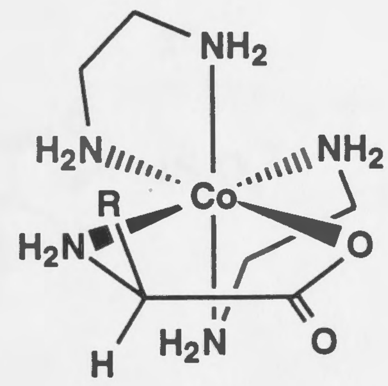



Figure 9: Reduction of α-imino acids by $BD_4^-$.

In all instances of reduction involving bis-ethane diamine complexes, the formation of the ΛR, ΔS diastereoisomers **43** were favoured over the ΛS, ΔR diastereoisomers **44**. These have proven to be the favoured isomers in the equilibrium mixture. It has been postulated that the existence of non-bonding steric interactions between the side chain of the amino acid and the ethane diamine chelate rings influences the equilibrium distribution of isomers. The isomer which minimises the interactions between the side chain of the amino acid and the co-ligands will be the most stable and most abundant form, Figure 10.[44,51]





**43**

ΛR, ΔS
most stable isomer

**44**

ΛS, ΔR
least stable isomer

It would be expected that increasing the bulk of the side chain would affect the eventual isomer distribution of the amino acid complex. However, reduction of the imine occurred by attack of the reducing agent at the more hindered side of the imino acidato ligand, giving rise to an excess of the ΛR, ΔS diastereoisomers and, with the exception of the glycine imine and valine imine complexes, the size of the side chain had virtually no effect on the isomer distribution of the resulting amino acid complexes; ΛR, ΔS : ΛS, ΔR = 6 : 4. When $[(en)_2Co(gly\text{-}im)]^{2+}$ was reduced by both $BD_4^-$ and by $S_2O_4^{2-}$ /$D_2O$/DCl, the resulting isomer distribution was ΛR, ΔS : ΛS, ΔR = 1 : 1. This is not entirely exceptional since the 'side chain' of glycine is a proton, and so steric constraints on these isomers are not as influential as they are in other cases. When $[(en)_2Co(val\text{-}im)]^{2+}$ was reduced by $S_2O_4^{2-}$ the isomer distribution was ΛR, ΔS : ΛS, ΔR = 4.2 : 1.0. One could argue that the bulk of the methyl groups on the β-carbon of the imino acid impose greater steric constraints on the reaction than the methylene β-carbon of the side chains of most of the other imino acid complexes. The $[(en)_2Co(phenyl\text{-}gly\text{-}im)]^{2+}$ complex was reduced with $S_2O_4^{2-}$ to see whether the presence of the benzene ring affected the stereoselectivity of the reduction, but the distribution of isomers was approximately ΛR, ΔS : ΛS, ΔR = 6 : 4. Molecular (Dreiding) models of the complex implied that the steric crowding about the region of the α-carbon would ~~in fact~~

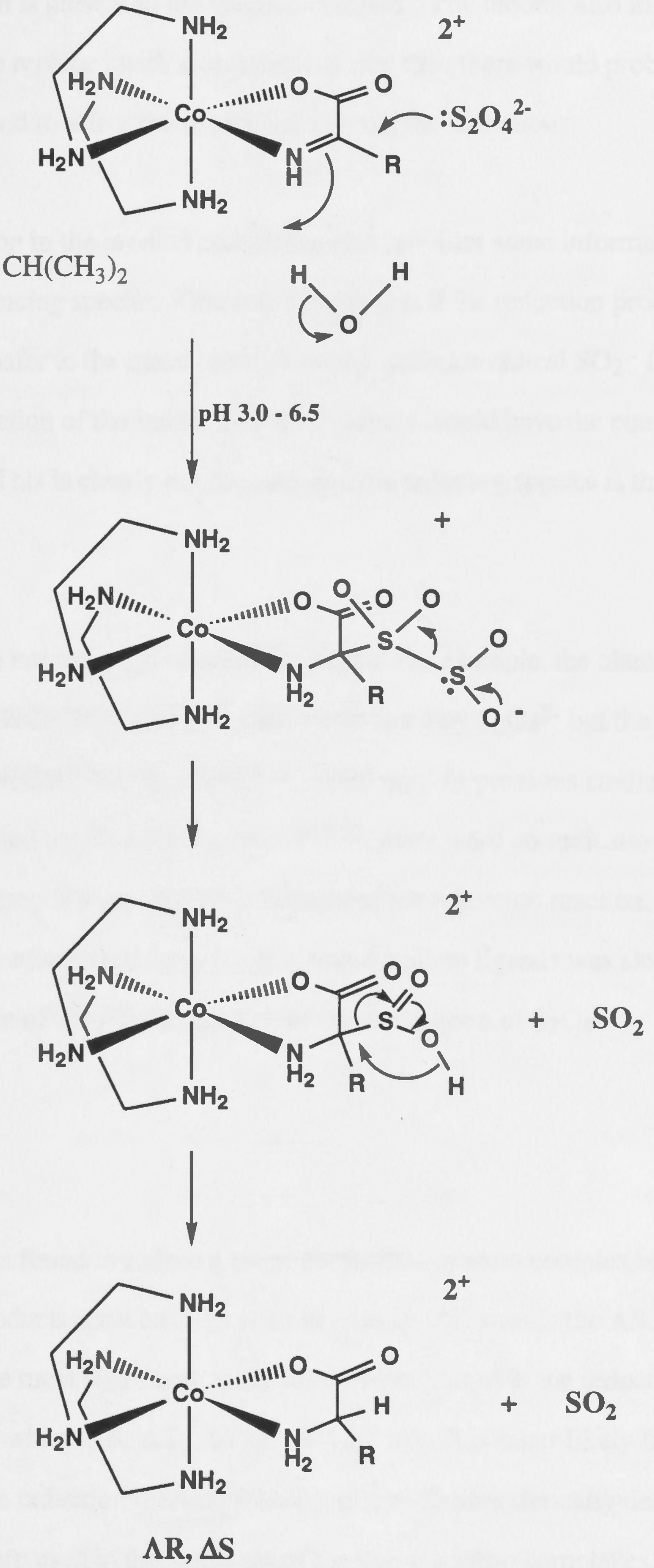



Figure 10: Stereoselective Reduction of $[(en)_2Co(val\text{-}im)]^{2+}$ by $S_2O_4^{\ 2-}$.

be somewhat less than is present in the valinato complex. The models also implied that if the benzene ring were replaced with a cyclohexane ring then there would probably be sufficient steric demand to affect the stereoselectivity of the reduction.

The isomer distribution in the product complexes also provides some information about the identity of the reducing species. One could argue that if the reduction process involved electron transfer to the metal centre from the sulfinate radical $SO_2^{\cdot -}$ followed by fast transfer and reduction of the imine, then the products would have the equilibrium isomer distribution. This is clearly not the case and the reducing species is thus most likely to be $S_2O_4^{2-}$.

Sodium dithionite did not reduce N-alkyl imino acids. For example, the alaninato complex, $[(en)_2Co(\mathbf{NH}C(CH_3)COO)]^{2+}$ could be reduced by $S_2O_4^{2-}$ but the N-methyl analogue, $[(en)_2Co(\mathbf{N(CH_3)}C(CH_3)COO)]^{2+}$, could not. In previous studies involving reductions of imines and pyridine derivatives[13-19,22], there were no indications of involvement of the nitrogen atom in the mechanism of the reduction reaction. It is most likely that the rate of reduction of these N-alkyl imino acidato ligands was slow compared to the rate of reduction of Co(III) and the rate of decomposition of $S_2O_4^{2-}$.

## *CONCLUSION.*

Sodium dithionite was found to reduce a range of α-imino acidato complexes. The distribution of the products showed a small stereoselectivity towards the ΛR, ΔS diastereoisomers. The most significant selectivity was exhibited in the reduction of $[(en)_2Co(val\text{-}im)]^{2+}$, where ΛR, ΔS : ΛS, ΔR = 4.2 : 1.0. It is most likely that the stereoselectivity of the reduction reaction would improve if more sterically demanding tetraamine ligands were used in the synthesis of the imino acidato complexes.

The reduction occurs best at a pH of 3.0 - 5.5; when the pH rises to 6.5 or higher reduction of Co(III) becomes a problem. This is in direct contrast to the conditions ( pH ~ 10) under which sodium borohydride becomes a useful reducing agent, so it is a valuable addition to the tools used to synthesise new amino acid complexes.

Unlike borohydride, dithionite ion does not reduce N-alkyl amino acids. This seems to be a result of the relative rate of reduction of the amino acid compared to the rate of reduction of Co(III) and the decomposition of dithionite, rather than a characteristic of the mechanism of the reaction.

A mechanism of the reduction of the imino acids has been proposed which accounts for the observations made during the course of the study, and concurs with mechanisms proposed for earlier reductions of related compounds.

## Experimental

### *INSTRUMENTS, REAGENTS AND ANALYSES*

Nuclear magnetic resonance spectra of the complexes dissolved in $D_2O$ or 0.1M DCl were acquired using a Varian Instruments Gemini 300 NMR spectrometer. Chemical shifts in $^1H$ nmr spectra are reported relative to sodium trimethylsilylpropane sulfonate (NaTPS), 0.00ppm. Chemical shifts in $^{13}C$ nmr are reported relative to dioxane, 67.4 ppm. Multiplicities of signals in the $^1H$ nmr spectra are indicated by the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br). Optical density changes were monitored during kinetics experiments using a thermostated, hand-operated, stopped-flow mixer which was fitted into the cell compartment of a Cary 118C spectrophotometer. Optical rotary dispersion spectra (ORD) were obtained from a Perkin-Elmer P22 spectropolarimeter. IR spectra were obtained using KBr discs in a Perkin Elmer PE 1800 FTIR spectrophotometer by Mr

Denes Bogsani. Elemental microanalyses were performed by the ANU Microanalytical Service.

Most solvents and basic chemicals used for syntheses were analytical reagent grade. Commercial MMM$CF_3SO_3H$ was distilled before use. Dmf was dried over $CaSO_4$ before use. Ion exchange chromatography was performed with analytical grade Dowex 50Wx2 ($H^+$ form, 200 - 400 mesh, Bio-Rad) or SP Sephadex C25 ($Na^+$ form, Pharmacia). Complexes present in the collected eluents were recovered by evaporation (~ 20 τ) on a Büchi rotary evaporator, with a water bath temperature of less than 40 °C. L R grade $Na_2S_2O_4$ (AJAX) was used in all experiments.

***SYNTHESES***

The complexes [(en)$_2$Co(gly-im)]($ClO_4$)$_2$,[42] [(en)$_2$Co(ala-im)]$Cl_2$,[39] [(en)$_2$Co(val-im)]$Cl_2$,[39] [(en)$_2$Co(val-im)](ClO4)$_2$,[39] [(en)$_2$Co(N-methyl val-im)]$Cl_2$,[52] [(en)$_2$Co(met-im)]$Cl_2$,[39] [(en)$_2$Co(tyr-im)]$Cl_2$,[39], [(en)$_2$Co(lys-im)]$Cl_2$,[39] [(en)$_2$Co(phenyl-gly-im)]($ClO_4$)$_2$,[41] [(en)$_2$Co(S-methyl-met-im)]$Cl_2$,[41] [(en)$_2$Co(vinyl-gly-im)]($ClO_4$)$_2$,[41] [(en)$_2$Co(NHC(CH=CH($C_6H_4OH$))COO)]$Cl_2$,[40] [(en)$_2$Co(pro-im)]$Cl_2$,[39] [(en)$_2$Co(pip-im)]$Cl_2$,[39] [($NH_3$)$_4$Co(ala-im)]$Cl_2$,[37] [($NH_3$)$_4$Co(sar-im)]$Cl_2$,[37] [($NH_3$)$_4$Co(glu-im)]$Cl_2$,[37] [($NH_3$)$_4$Co(phenyl-gly-im)]($ClO_4$)$_2$,[37] [(bipy)$_2$Co(ala-im)]$Cl_2$,[41] and [Co(($CH_3$)$_2$ - diimino-sar)]$ZnCl_4$.Cl[43] were prepared using previously established syntheses.

If it was necessary to separate the diastereoisomers of the complex this was done by adsorbing a small quantity of the complex (~ 50 -100 mg) on a Sephadex column (4.0 x 45.0 cm) and eluting the isomers with 0.05 M sodium citrate. The complexes were desalted on small beds of Dowex prior to analysis by $^1$H nmr spectrometry.

***Synthesis of (p)-[trenCo(ala)]Cl$_2$***

The ligand tren was isolated from crude trien as its hydrochoride salt.[53] Hydrochloric acid (36%, 400 cm$^3$) was added, in 10 - 20 cm$^3$ portions, to an ice-cold solution of vigorously stirred, crude trien (400 cm$^3$) in ethanol (1500 cm$^3$). Once all the acid had been added and the first white crystals had begun to precipitate from solution, the mixture was stored at 5 $^o$C overnight. The white crystals that precipitated were collected and recrystallised from water by the addition of ethanol (35 g).

To synthesise the complex, $H_2O_2$ (15 cm$^3$) was added dropwise to a stirred slurry of tren.3HCl (10.10 g), Co(CO$_3$).3(Co(OH)$_2$).H$_2$O (4.25 g), L-alanine (7.10 g), NaOH (3.25 g), activated charcoal (1.0 g) and $H_2O$ (70 cm$^3$). The resulting mixture was stirred for an hour before filtering and diluting it to 300 cm$^3$. It was then passed down a Dowex column (7 x 17 cm) and the adsorbed material washed with water and with 0.5 M HCl before being eluted from the column with 1 M HCl. The faint traces of orange material that eluted first were not collected. A second fraction, of poorly resolved pink and orange coloured complexes, did not contain an amino acid complex ($^1$H nmr spectrophotometry) and was discarded. The final fraction contained the complex [trenCo(alaninato)]$^{2+}$. The acid was removed by rotary evaporation and the residue recrystallised from water by the addition of ethanol. The resulting mustard-orange crystals were collected by vacuum filtration, washed with a little ice-cold water/ethanol and then again with acetone before air drying (2.7 g, 64%). Analysis calculated for [CoC$_9$H$_{24}$N$_5$O$_2$Cl$_2$]: Co, 16.18; C, 29.68; H, 6.64; N, 19.23; Cl, 16.18. Found: Co, 16.2; C, 29.7; H, 7.0; N, 19.2; Cl, 18.8. $^1$H nmr (0.1 M HCl): $\delta$ 4.4 - 6.2 (br, tren-NH$_2$); 2.8 - 3.8 (br, 12H, tren-CH$_2$); 1.49 (d, 3H, -CH$_3$). $^{13}$C nmr (0.1 M HCl): $\delta$ 185.2 (COO), 62.5, 59.9, 45.1, 46.2, 45.6 (2C), (tren CH$_2$), 25.8 (CH$_3$). Visible spectrum ($\lambda_{max}$, $\varepsilon_{max}$ in 0.05 M acetate buffer, pH 4.06): 476.1 nm, 107 M$^{-1}$cm$^{-1}$.

***Synthesis of (p)-[trenCo(ala-im)]Cl$_2$***

The triflate salt, (p)-[trenCo(ala)]($CF_3SO_3$)$_2$, was prepared in the usual way[49] by dissolving the corresponding chloride salt in anhydrous triflic acid and bubbling a stream of nitrogen through the resulting solution to drive off hydrochloric acid as it formed. The complex was precipitated by pouring the solution into vigorously stirred diethyl ether. The sticky solid was triturated with additional diethyl ether, collected by vacuum filtration and stored under vacuum, in a desiccator.

A portion of this material (2.00 g) was dissolved in dmf (15 cm$^3$) and the resulting solution chilled to 0 $^o$C. Thionyl chloride (10 cm$^3$) was added dropwise over ten minutes to the vigorously stirred solution. Once addition was complete the solution was stirred a further ten minutes at 0 $^o$C and then for a further 30 minutes at 25 $^o$C. The reaction was quenched by carefully pouring the solution into a stirred slurry of ice and water (1 dm$^3$). Once the ice had melted the aqueous solution was filtered to remove coagulated sulphur, diluted to about 1.5 dm$^3$ and the complexes were adsorbed on to a Dowex column (5 x 15 cm). The adsorbed material was washed with water and 0.5 M HCl before being eluted with 3 M HCl. A single orange band, which proved to be the product, was collected. The solvent was removed by rotary evaporation and the residue was recrystallised from water by the addition of ethanol. The mustard-orange crystals were collected by vacuum filtration, washed with ice cold water/ethanol solution, ethanol and acetone before being dried over silica (1.04 g, 85%). Analysis calculated for [$CoC_9H_{22}N_5O_2Cl_2$].$H_2O$: Co, 15.50; C, 28.44; H, 6.36; N, 18.42; Cl, 18.65. Found: Co, 15.7 ; C, 29.1; H, 6.9; N, 18.8; Cl, 18.2. $^1$H nmr (0.1 M HCl): $\delta$ 4.5 - 6.0 (br, tren-$NH_2$); 2.8 - 3.8 (br, 12H, tren-$CH_2$); 2.52 (s, 3H, -$CH_3$). . $^{13}$C nmr (0.1 M HCl): $\delta$ 186.0 (COO), 174.4 (C=N), 62.3, 59.7, 45.0, 46.2, 45.6 (2C), (tren $CH_2$), 23.5 ($CH_3$). . Visible spectrum ($\lambda_{max}$, $\varepsilon_{max}$ in 0.05 M acetate buffer, pH 4.06): 476.1 nm, 118 $M^{-1}cm^{-1}$.

## *REDUCTION OF [$N_4Co(NHC(R)COO)]^{2+}$*

### *Reduction of [(en)$_2$Co(val -im)](ClO$_4$)$_2$*

[(en)$_2$Co(val imine)](ClO$_4$)$_2$ (0.100 g, 2.03 x $10^{-4}$ moles) was dissolved in buffer solution (0.5 M, 10 cm$^3$) at 25 $^o$C. Sodium dithionite (0.195 g, 1.02 x $10^{-3}$ moles) was added to the stirred solution. After 30 minutes the reaction was quenched by diluting it with water (~ 90 cm$^3$) and passing the solution down a column of Dowex ion exchange resin (2.5 x 7.0 cm) with the aid of suction provided by a water pump. The adsorbed material was washed with water and with 0.5 M HCl (to remove traces of $Co^{2+}$) before being eluted from the column as a single fraction with 3 or 4 M HCl. The solvent was removed by rotary evaporation and the residual solid dissolved in $D_2O$ or 0.5 M DCl for $^1$H nmr spectrophotometry. $^1$H nmr ($D_2O$): $\delta$ 4.2 - 5.4 (br, en-NH$_2$); 3.73 (d, 1H, $\Lambda$R, $\Delta$S-NH$_2$-**CH**-); 3.57 (d, 1H, $\Lambda$S, $\Delta$R-NH$_2$-**CH**-); 2.6 - 2.8 (br, 8H, en-CH$_2$); 2.35 (m, 1H, -**CH**(CH$_3$)$_2$; 1.14 (d), 0.94 (d) (6H, $\Lambda$R, $\Delta$S-**CH**(CH$_3$)$_2$); 1.14(d), 0.99 (d) (6H, $\Lambda$S, $\Delta$R-**CH**(CH$_3$)$_2$).

Variations to this procedure included: concentration of substrates, temperature (0 $^o$C, 25 $^o$C), pH (3.0, 4.1 (acetate); 5.5, 6.5, 7.5 (Bis Tris); 8.5 (HEPES); 9.5 (carbonate)) and an air or $N_2$ atmosphere. If the reaction was done in $D_2O$ the solution remained unbuffered; the pH of the unbuffered solution was approximately 4.5. The related complexes [(NH$_3$)$_4$Co(val-im)]Cl$_2$ and [(en)$_2$Co(N-methyl-val-im)]Cl$_2$ were reacted with dithionite at pH 4.1 using the procedure described above.

### *Reduction of [(en)$_2$Co(ala -im)]Cl$_2$*

In an analogous method to that described above, [(en)$_2$Co(ala-im)](ClO$_4$)$_2$ (0.100 g, 2.97 x $10^{-4}$ moles) was dissolved in acetate buffer solution (pH 4.1, 0.5 M, 10 cm$^3$) at 25 $^o$C. Sodium dithionite (0.286 g, 1.49 x $10^{-3}$ moles) was added to the stirred solution. After 30 minutes the reaction was quenched by diluting it with water (~ 90 cm$^3$) and passing the solution down a column of Dowex ion exchange resin (2.5 x 7.0 cm) with the aid of suction provided by a water pump. The adsorbed material was washed with water and with 0.5 M HCl (to remove traces of $Co^{2+}$) before being eluted from the column as a single fraction with 3 or 4 M HCl. The solvent was removed by rotary evaporation and the residual solid dissolved in $D_2O$ or 0.5 M DCl for $^1H$ nmr spectrophotometry. $^1H$ nmr ($D_2O$): $\delta$ 4.1 - 5.8 (br, en-$NH_2$); 3.85 (q, 1H, ΛR, ΔS-$NH_2$-**CH**-); 3.71 (q, 1H, ΛS, ΔR-$NH_2$-**CH**-); 2.6 - 3.0 (br, 8H, en-$CH_2$); 1.51 (d, 3H, ΛR, ΔS-$CH_3$); 1.49 (d, 3H, ΛS, ΔR-$CH_3$).

This procedure was also used for the complexes $[(NH_3)_4Co(ala\text{-}im)]Cl_2$, $[(NH_3)_4Co(N\text{-}methyl\text{-}ala\text{-}im)]Cl_2$, $[(bipy)_2Co(ala\text{-}im)]Cl_2$, and (p)-$[trenCo(ala\text{-}im)]Cl_2$.

*Reduction of $[(en)_2Co(gly\text{-}im)]Cl_2$*

$[(en)_2Co(gly\ imine)](CF_3SO_3)_2$, (0.100 g, 1.8 x $10^{-4}$ moles) was dissolved in acetate buffer (0.5 M, pH 4.1, 10 $cm^3$). Nitrogen was bubbled through the solution for 20 minutes before sodium dithionite (0.035 g, 1.8 x $10^{-4}$ moles) was added, with vigorous stirring. After 30 seconds the reaction was quenched as described for reduction of $[(en)_2Co(ala\ imine)]Cl_2$. $^1H$ nmr ($D_2O$): $\delta$ 4.2 - 5.8 (br, en-$NH_2$); 3.75 (s, 2H, $NH_2$-$\mathbf{CH_2}$); 2.6 - 3.0 (br, 8 H, en-$CH_2$).

***Reductions of $[N_4Co(imino\ acidato)]^{2+}$ by $S_2O_4^{2-}$***

The complexes$[(en)_2Co(tyr\text{-}im)]Cl_2$, $[(en)_2Co(lys\text{-}im)]Cl_2$, $[(en)_2Co(phenyl\text{-}gly\text{-}im)](ClO_4)_2$, $[(en)_2Co(S\text{-}methyl\text{-}met\text{-}im)]Cl_2$, $[(en)_2Co(vinyl\text{-}gly\text{-}im)](ClO_4)_2$, $[(en)_2Co(NHC(CH{=}CH(C_6H_4OH))COO)]Cl_2$, $[(en)_2Co(pro\text{-}im)]Cl_2$, $[(en)_2Co(pip\text{-}im)]Cl_2$, $[(NH_3)_4Co(sar\text{-}im)]Cl_2$, $[(NH_3)_4Co(glu\text{-}im)]Cl_2$, and $[(NH_3)_4Co(phenyl\text{-}gly\text{-}im)](ClO_4)_2$ were reacted with dithionite in the manner described for the reduction of $[(en)_2Co(ala\text{-}im)]Cl_2$.

$[Co((CH_3)_2$ - diimino-sar)]$ZnCl_4.Cl$ (50 mg) was dissolved in a mixture of $D_2O$ (0.70 $cm^3$) and acetate buffer (0.5 M, pH 4.1, 0.4 $cm^3$). Portions of sodium dithionite (4 x 40 mg) were added to this solution every 2 hours. The reaction was monitored by $^{13}C$ nmr spectrophotometry. No conversion of imine to amine was detected.

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# CHAPTER 3

## Synthesis of Labelled Amino Acids as Probes of Structure and Metabolic Function

## Introduction

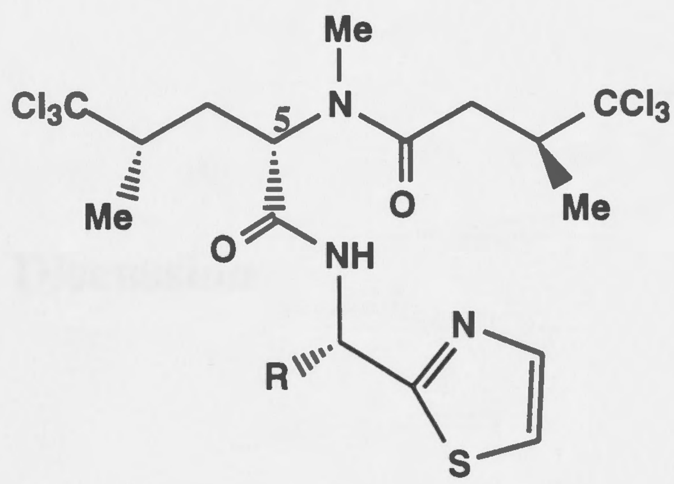
This chapter demonstrates the way in which α-imino acid complexes of Co(III) may be used to synthesise a range of regioselectively deuteriated amino acids that are to be used as probes of the structure and biosynthesis of tropical marine metabolites.

Marine natural products chemistry involves the isolation and characterisation of metabolites from a huge variety of plant and animal species.[1] The structure and biosynthesis of such natural products provide information about their purpose (such as chemical defence[2]), their origins (whether synthesised by the organism or by a symbiont[3]) and about the interaction between the organism and its environment (such as an organism's response to changing nutrient levels[1]). Whilst the structure of a newly isolated marine metabolite is determined during its identification and characterisation, there have been far fewer studies of the biosyntheses of these compounds.

Much of the interest in marine natural products involves their potentially useful bioactivity, particularly their antimicrobial and anticancer activity.[1,4] However, these compounds tend to exist in very low concentrations in the organism and harvesting sufficient quantities of the species for clinical evaluation of the metabolite may seriously deplete the wild population.[5] In these circumstances it is important to determine how the organism synthesises the metabolite so that it might be replicated and/or modified in the laboratory.

Mapping the biosynthetic pathway that leads to the metabolite of interest usually begins with identification of precursors ('building blocks'), such as amino acids, that form its structure. This requires precursors that are labelled with radioactive[6] (commonly $^{14}C$) or stable[7] (such as $^{2}H$) isotopes. Incorporation of radio-labelled precursors can be detected even at low levels of incorporation. However, during analysis the metabolite is completely destroyed, whereas the use of stable isotope labelled precursors allows analysis of the isolated metabolite by nmr spectrometry[1] and mass spectroscopy.

A team led by Dr Mary Garson of the University of Queensland has isolated a number of bioactive metabolites from the tropical marine sponge *Dysidea herbacea*. These include polychlorinated amino acid derivatives, **1**, which are iodide transport inhibitors or have antihypertensive activity. $^{14}$C-leucine and valine have been incorporated into **1d** and the incorporation levels were considered high enough to make incorporation experiments with $^{2}$H-leucine and -valine feasible.[8]




**1**

**a**: R = Me; Dysidenin
**b**: R = H; 13-Demethyldysidenin
**c**: R = Me; epimer at C-5; Isodysidenin
**d**: R = H; epimer at C-5; 13-Demethylisodysidenin

Figure 1: Metabolites isolated from *Dysidea herbacea*, a tropical marine sponge.

Co(III) imino acidato complexes are suitable reagents for the synthesis of regioselectively labelled metabolites such as these. The previous chapter described how reduction of coordinated α-imino acids with $BD_4^-$ or $S_2O_4^{2-}/D_2O$ yielded the corresponding 2-deuterio- valine and alanine, Figure 2. Previous work has noted the ease with which protons on the β-carbon atom of the imino acid may be exchanged in aqueous, basic solution.[9a] A range of deuteriated amino acids was synthesised using both of these reactions for the incorporation experiments described above.

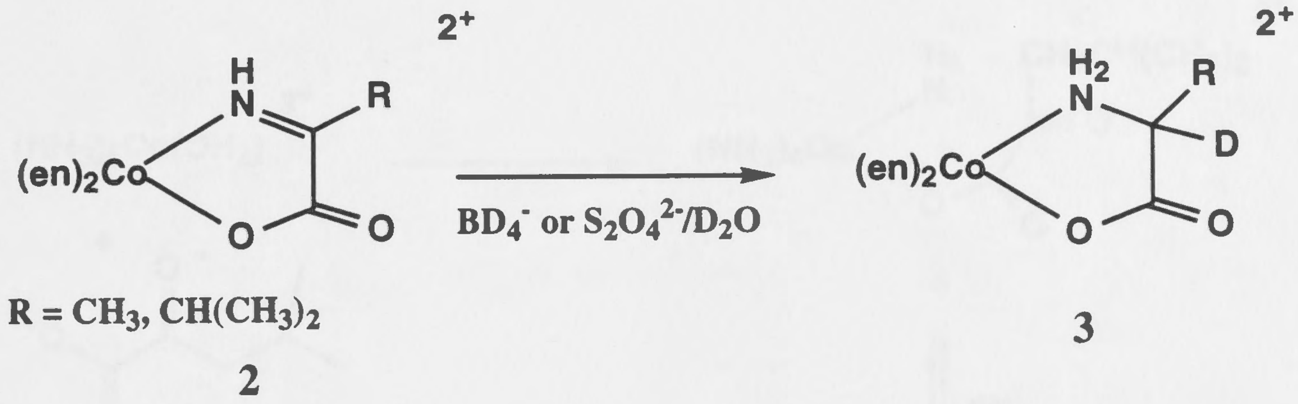


Figure 2: Labelling of amino acids with deuterium by reduction of the corresponding imino acids with $BD_4^-$ or $S_2O_4^{2-}/D_2O$.

## Results and Discussion

### *SYNTHESES*

#### *Regioselective Deuteriation of Leucine*

The precursor $[(NH_3)_4Co(leu\text{-}im)]^{2+}$, **7**, was synthesised by intramolecular Schiff base condensation between coordinated 4-methyl-α-oxopentanoate and ammonia, Figure 3. This methodology has previously been used to synthesise related complexes.[9b] The $^1H$ nmr spectrum of **7** is reproduced in Figure 4a. The relevant signals are: 4.1, 3.6, 3.3 (4 x $NH_3$), 2.95 (β-$CH_2$), 2.13 (γ-CH), 0.99, 0.98 (2 x $CH_3$) ppm.

The isolated complex was used to synthesise complexes of 2-deuterioleucine (**8**), 3, 3-dideuterioleucine (**10**) and 2, 3, 3- trideuterioleucine (**11**), Figure 5. Reductions involving $Na_2S_2O_4$ and $D_2O/H_2O$ were complicated by the presence of the sulfinate intermediate which led to small quantities of unlabelled amino acid and/or traces of elemental sulphur contaminating the isolated amino acids through its destruction in aqueous conditions. As a

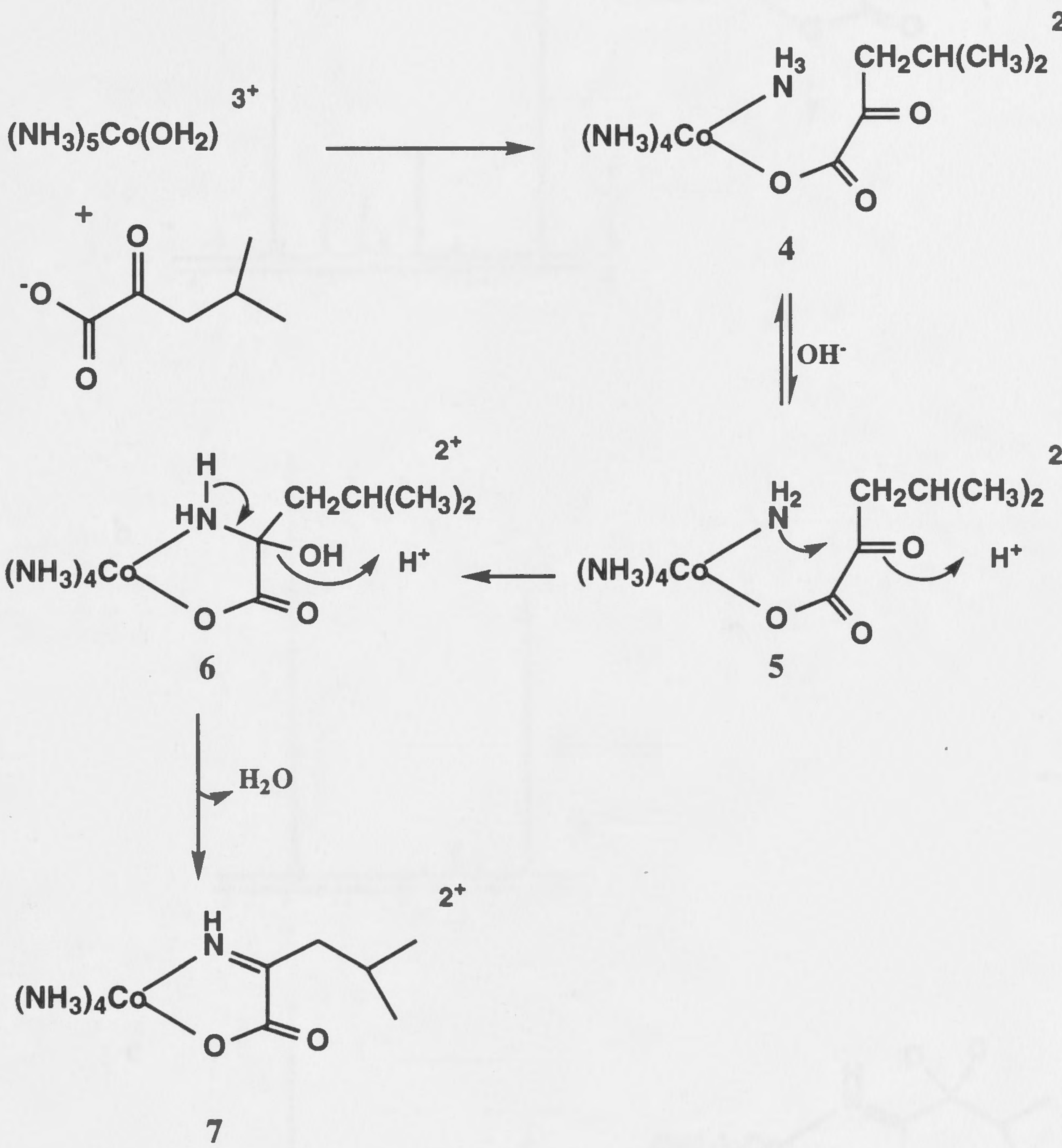



Figure 3: Synthesis of $[(NH_3)_4Co(leu\text{-}im)]^{2+}$

consequence the reduction reactions described here used $NaBH_4$ or $NaBD_4$ as the reducing agent. Complex **8** was obtained by reducing **7** with $BD_4^-$ under mild, basic conditions (pH 10.0).[9] Complexes **10** and **11** required the synthesis of the intermediate **9**, obtained after dissolving **7** in a $CO_3^{2-}/DCO_3^-$ buffer (pH 10.5). The exchange of the protons on the β carbon (~ 2.6 ppm at pH 10.0) was monitored by $^1H$ nmr spectroscopy (complete exchange required about 10 hours), Figure 4b, 4c. Sodium -borodeuteride or -borohydride was then added to the solution, resulting in **10** and **11** respectively.

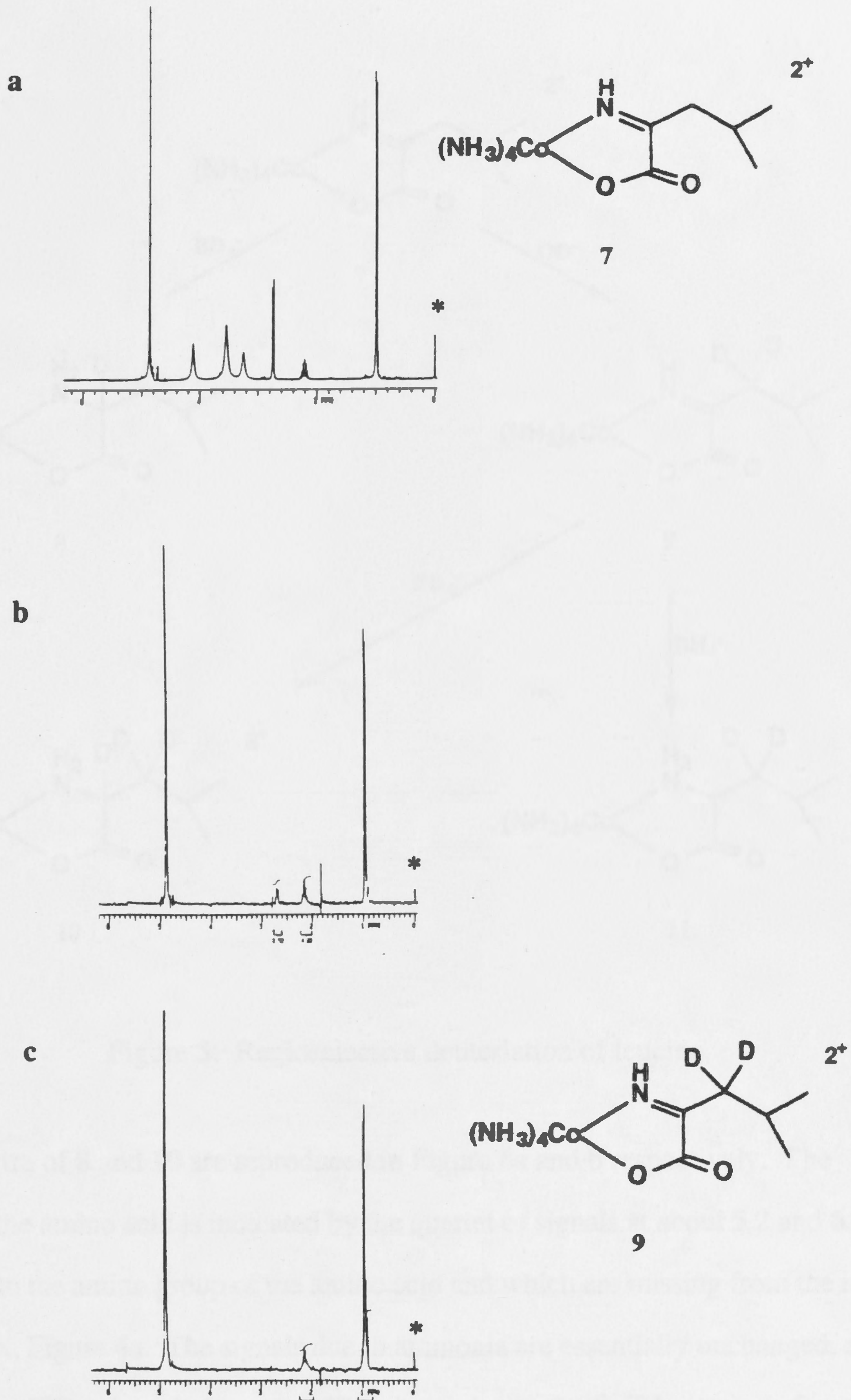



Figure 4: Exchange of protons for deuterium on the β-carbon of $[(NH_3)_4Co(\text{leu-im})]^{2+}$.

**a**: t = 0 min. **b**: t = 55 min. **c**: t = 600 min.

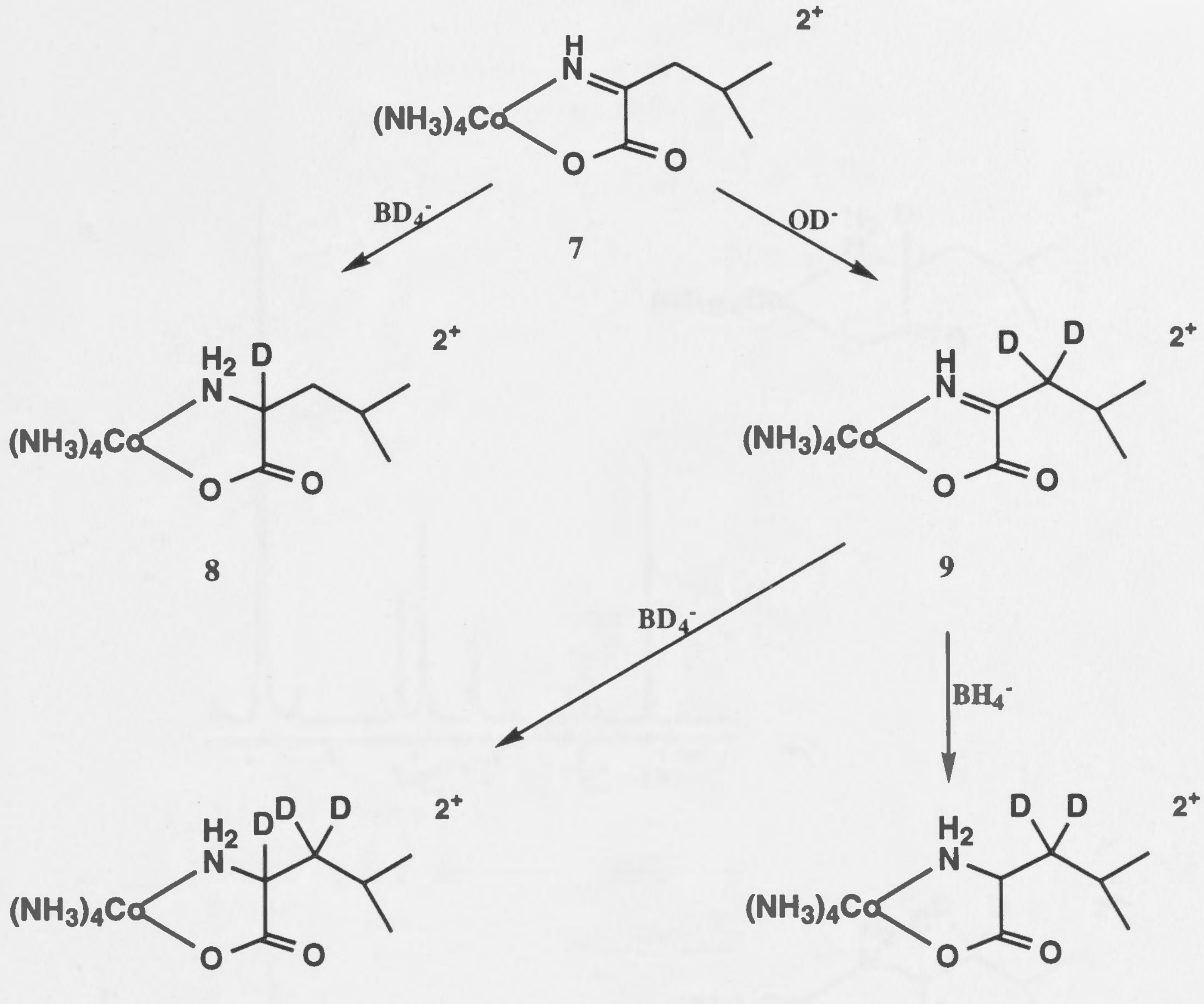



Figure 5: Regioselective deuteriation of leucine.

$^1H$ nmr spectra of **8** and **10** are reproduced in Figure 6a and b respectively. The presence of the amino acid is indicated by the quartet of signals at about 5.2 and 6.2 ppm that are due to the amino group of the amino acid and which are missing from the imino acid complex, Figure 4a. The signals due to ammonia are essentially unchanged, as are those of the $\gamma$-CH and methyl groups. The signal due to the $\beta$-$CH_2$ group is found at 1.88 ppm in the spectrum of **8** but is absent in that of **10** because the protons on this carbon have been exchanged for deuterium. The signal due to the $\beta$-$CH_2$ has an ABX coupling pattern due to the $\beta$-$CH_2$ protons and the $\gamma$-CH protons. The proton on the $\alpha$-CH of **11** (not shown) appears at 3.72 ppm.

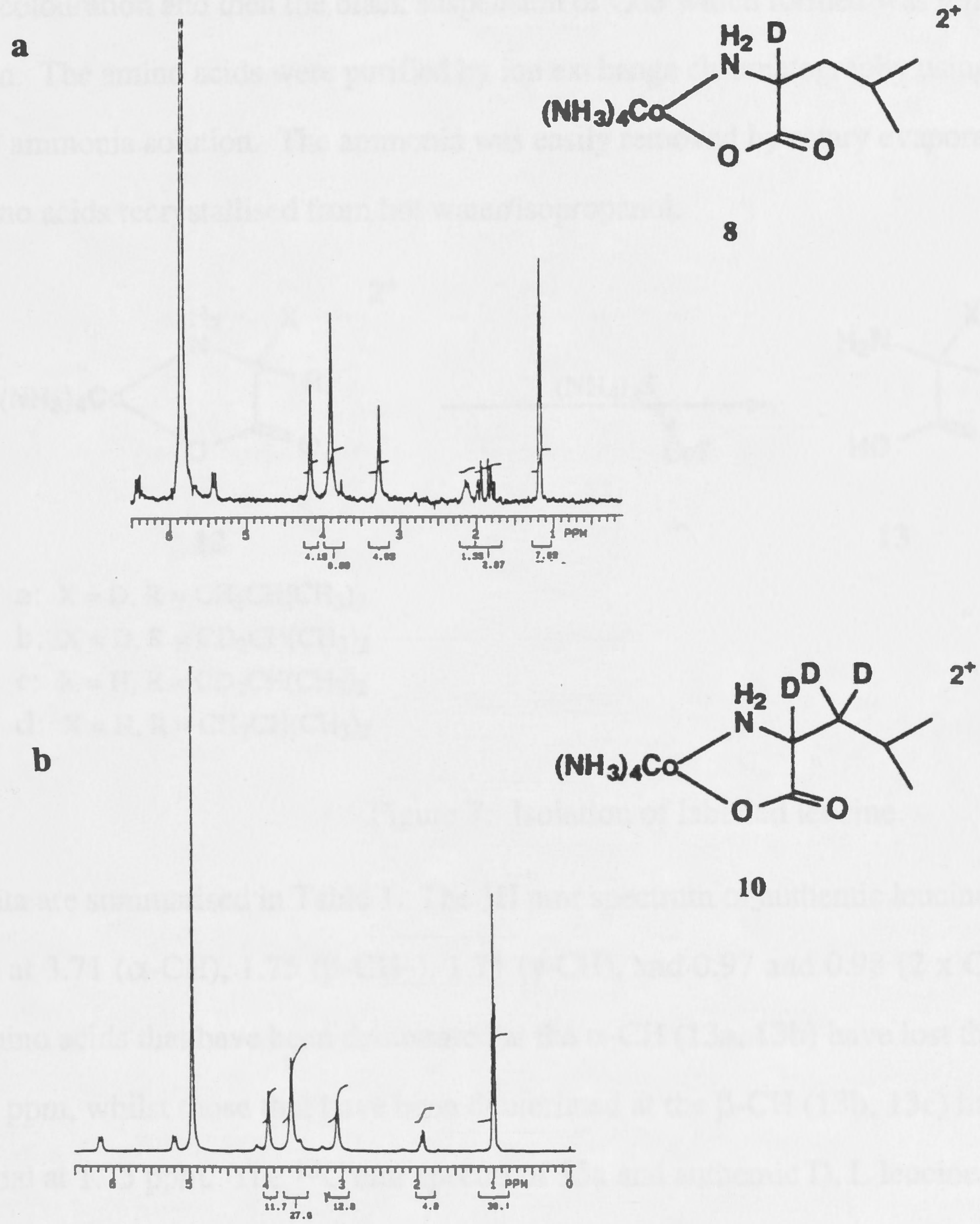
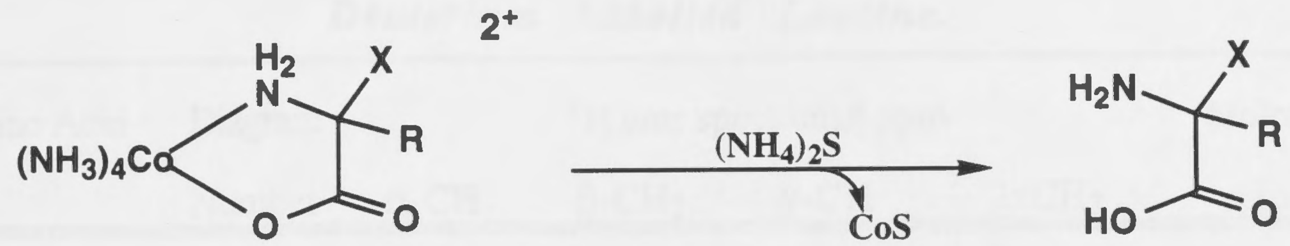



Figure 6: $^1$H nmr spectra (0.1M DCl, *NaTPS) of Co(III) complexes of regioselectively deuteriated leucine.

These complexes (**8, 10, 11**) were not isolated from solution; instead the amino acids were removed from the metal centre by reaction with ammonium sulfide, Figure 7. The sulfide was added to solutions of the complexes to the point at which the solutions lost all orange colouration and then the black suspension of CoS which formed was removed by filtration. The amino acids were purified by ion exchange chromatography using dilute (0.5 M) ammonia solution. The ammonia was easily removed by rotary evaporation and the amino acids recrystallised from hot water/isopropanol.




**12** **13**

**a**: X = D, R = $CH_2CH(CH_3)_2$
**b**: X = D, R = $CD_2CH(CH_3)_2$
**c**: X = H, R = $CD_2CH(CH_3)_2$
**d**: X = H, R = $CH_2CH(CH_3)_2$

Figure 7: Isolation of labelled leucine.

Nmr data are summarised in Table 1. The $^1H$ nmr spectrum of authentic leucine has signals at 3.71 (α-CH), 1.75 (β-$CH_2$), 1.71 (γ-CH), and 0.97 and 0.98 (2 x $CH_3$) ppm. The amino acids that have been deuteriated at the α-CH (**13a, 13b**) have lost the signal at 3.71 ppm, whilst those that have been deuteriated at the β-CH (**13b, 13c**) have lost the signal at 1.75 ppm. The $^{13}C$ nmr spectra of **13a** and authentic D, L leucine are reproduced in Figure 8. In the spectrum of authentic leucine (Figure 8a) there is a single signal at 56.3 ppm due to the α-carbon (α-CH). In the spectrum of **13a** (Figure 8b) this is replaced by a triplet ($J_{C\text{-}D}$ 24.5 Hz) because of coupling between the carbon and the deuterium atoms. The other relevant signals are 40.6 ppm (β-$CH_2$), 25.0 (γ-CH) and 22.8 and 21.7 ppm (2 x $CH_3$). The signal due to the carboxyl group (not shown) is at 188.0 ppm. In $^{13}C$ spectra of **13b** and **13c** the singlet due to the β-$CH_2$ is replaced by a complex and poorly resolved multiplet.[7a] The deuteriated leucine and valine molecules were also characterised by electrospray mass spectroscopy. The spectra for the leucine samples are reproduced in Figure 9. These show the molecular ions (amino acid + $H^+$) of **13a, 13b**, and **13c** at 133.0, 135.0 and 134.0 respectively. The molecular ion at 87.0, 89.0 and 88.0 is due to loss of the carboxylate group from the amino acid.

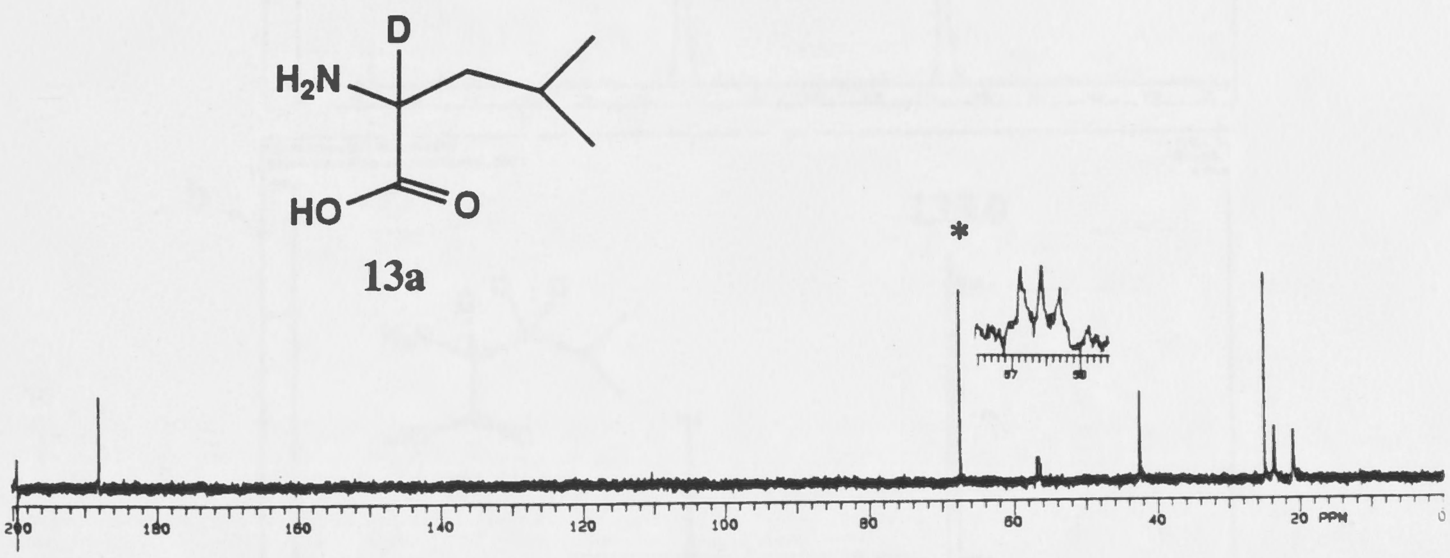
***Table 1: Comparison of $^1H$ and $^{13}C$ nmr Spectra of Authentic and Deuterium Labelled Leucine.***

| Amino Acid | Diagram Number | $^1H$ nmr spectrum,[a] ppm α-CH | β-$CH_2$ | γ-CH | 2x$CH_3$ | Molecular Ion |
|---|---|---|---|---|---|---|
| leucine | **13d** | 3.1 (t) | 1.75 (q) | 1.71 (m) | 0.97, 0.98 (d) | 132.2[b] |
| leucine-2-$^2H$ | **13a** | - | 1.75 (q) | 1.70 (m) | 0.97, 0.98 (d) | 133.0[c] |
| leucine-2,3,3-$^2H$ | **13b** | - | - | 1.70 (m) | 0.97, 0.98 (d) | 135.2[c] |
| leucine-3,3-$^2H$ | **13c** | 3.73 (s) | - | 1.69 (m) | 0.97, 0.98 (d) | 134.2[c] |
| 4-azaleucine | **17** | 3.42 (m) | 4.04 (q) | - | 2.95 | - |

| | | $^{13}C$ nmr spectrum,[d] ppm COOH | α-CH | β-$CH_2$ | γ-CH | 2x$CH_3$ |
|---|---|---|---|---|---|---|
| leucine | **13d** | 188.0 | 56.3 | 40.6 | 25.0 | 21.7, 22.8 |
| leucine-2-$^2H$ | **13a** | 187.9 | 56.8 (t)[e] | 42.4 | 24.9 | 21.0, 24.9 |
| leucine-2,3,3-$^2H$ | **13b** | 187.9 | 56.6 (t)[e] | 42.4 (m)[f] | 24.5 | 20.9, 24.5 |
| leucine-3,3-$^2H$ | **13c** | 187.8 | 56.5 | 42.3 (m)[f] | 24.5 | 20.9, 24.5 |
| 4-azaleucine | **17** | 176.4 | 59.5 | 49.9 | - | 43.4 |

[a]$D_2O$, NaTPS as internal standard. [b]Calculated (molecular weight + H)$^+$. [c]Electrospray ms ($CH_3CN$), ions are (amino acid + H)$^+$. [d]$D_2O$, dioxane as internal standard. [e]$J_{C\text{-}D}$ = 24.5 Hz. [f] $J_{C\text{-}D}$ could not be determined. s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

a




13a

b

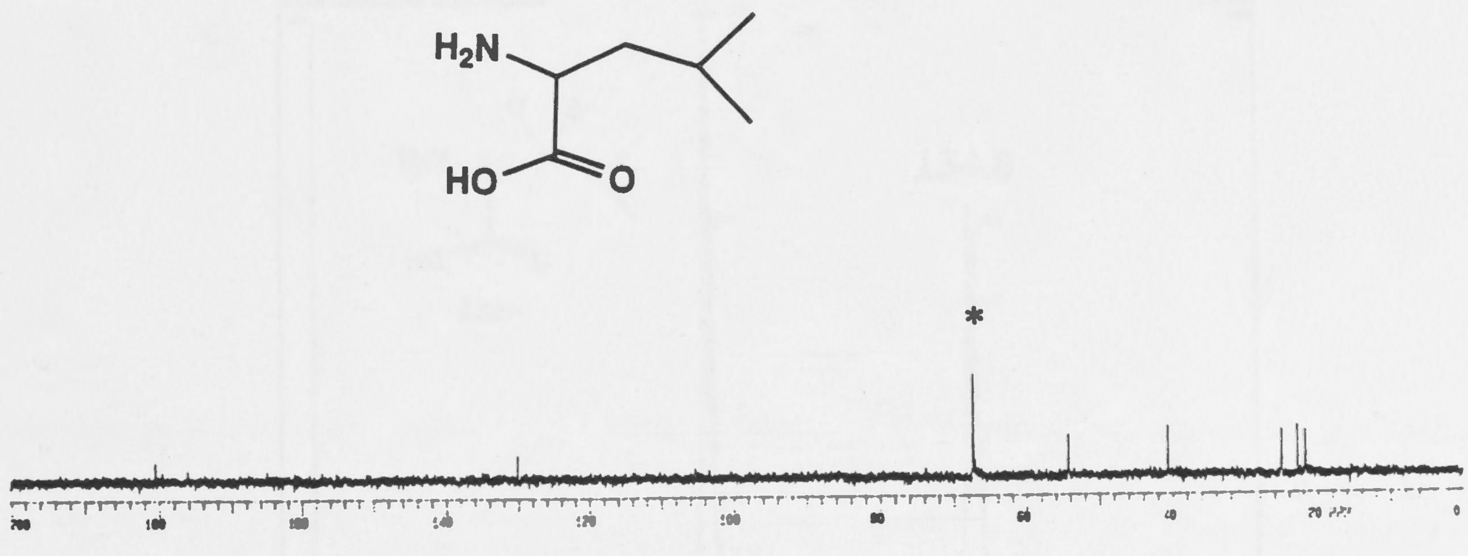



Figure 8: Comparison of the $^{13}C$ nmr spectrum ($D_2O$, *dioxane) of **13a** (leucine deuteriated at the α-carbon) with that of authentic (non deuteriated) leucine.

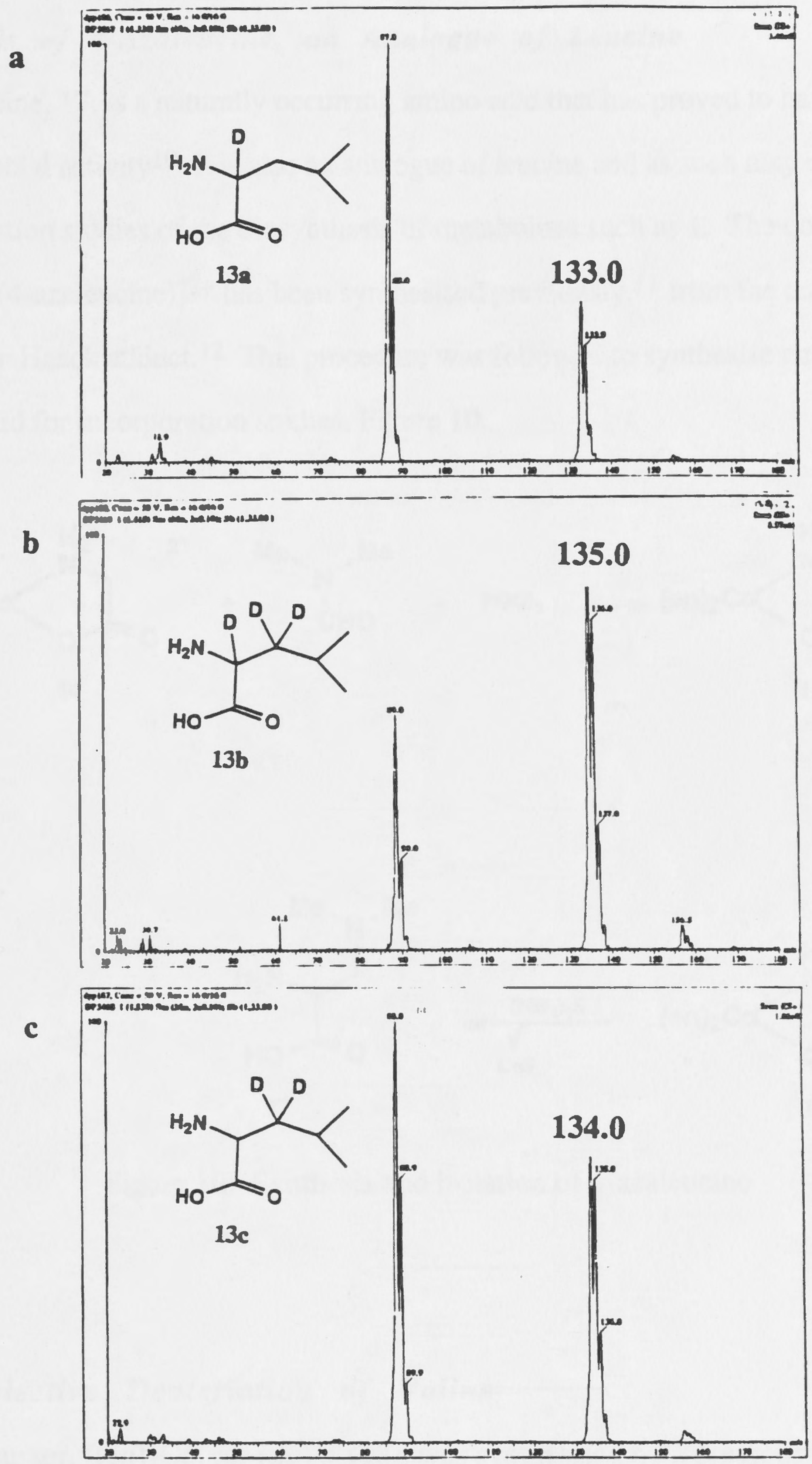



Figure 9: Electro-spray mass spectra of samples of regioselectively deuteriated leucine (in $CH_3CN$).

***Synthesis of 4-Azaleucine, an Analogue of Leucine***

4-azaleucine, **17**, is a naturally occurring amino acid that has proved to have potent antimicrobial activity[10] It is also an analogue of leucine and as such may also be used in incorporation studies of the biosynthesis of metabolites such as **1**. The complex $[(en)_2Co(4\text{-azaleucine})]^{2+}$ has been synthesised previously,[11] from the corresponding Vilsmeier-Haack adduct.[12] This procedure was followed to synthesise and isolate the amino acid for incorporation studies, Figure 10.

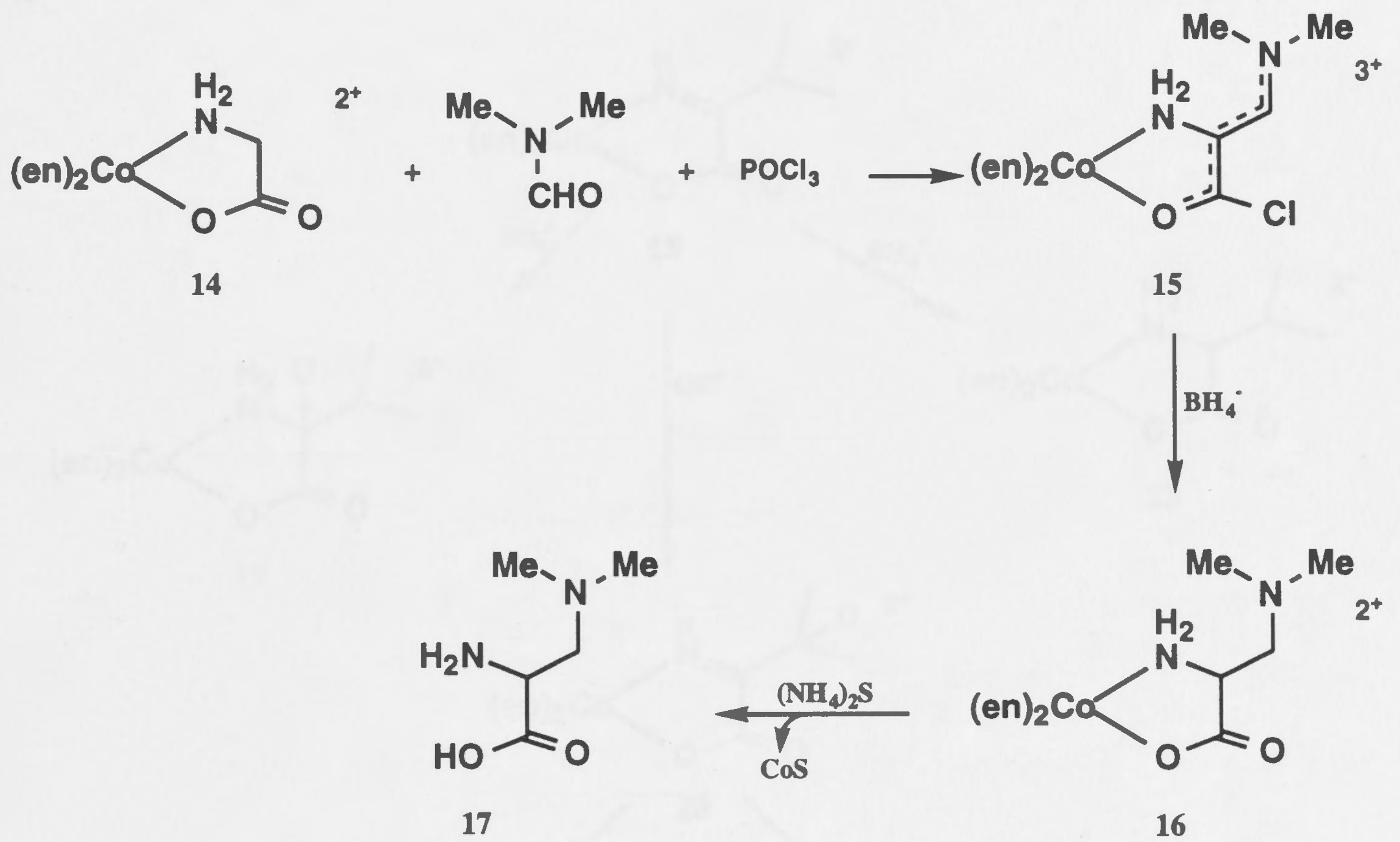



Figure 10: Synthesis and isolation of 4-azaleucine

***Regioselective Deuteriation of Valine***

The precursor, $[(en)_2Co(val\text{-}im)]^{2+}$ (**18**), was synthesised by a previously published synthesis.[13] The proton on the β-carbon was exchanged (**20**) by dissolving the complex in a $CO_3^{2-}/DCO_3^-$ buffer at pH 10.0 as described above for the corresponding leucine complexes. Reduction of **18** or **20** by sodium borodeuteride or borohydride yielded complexes of 2-deuteriovaline (**19**), 2, 3-deuteriovaline (**21**) and 3-deuteriovaline (**22**), Figure 11. $^1$H nmr

spectra of **18**, **21**, **22** and **23** are reproduced in Figure 12. The spectrum of the imino acid complex, Figure 12a, is relatively straightforward. The relevant signals are the multiplet at 3.18 ppm due to the β-CH and the doublets at 1.32 and 1.29 ppm due to the methyl groups. If the imine is reduced with $BH_4^-$ or $S_2O_4^{2-}$ (**23**), Figure 12d, the methyls' signals are

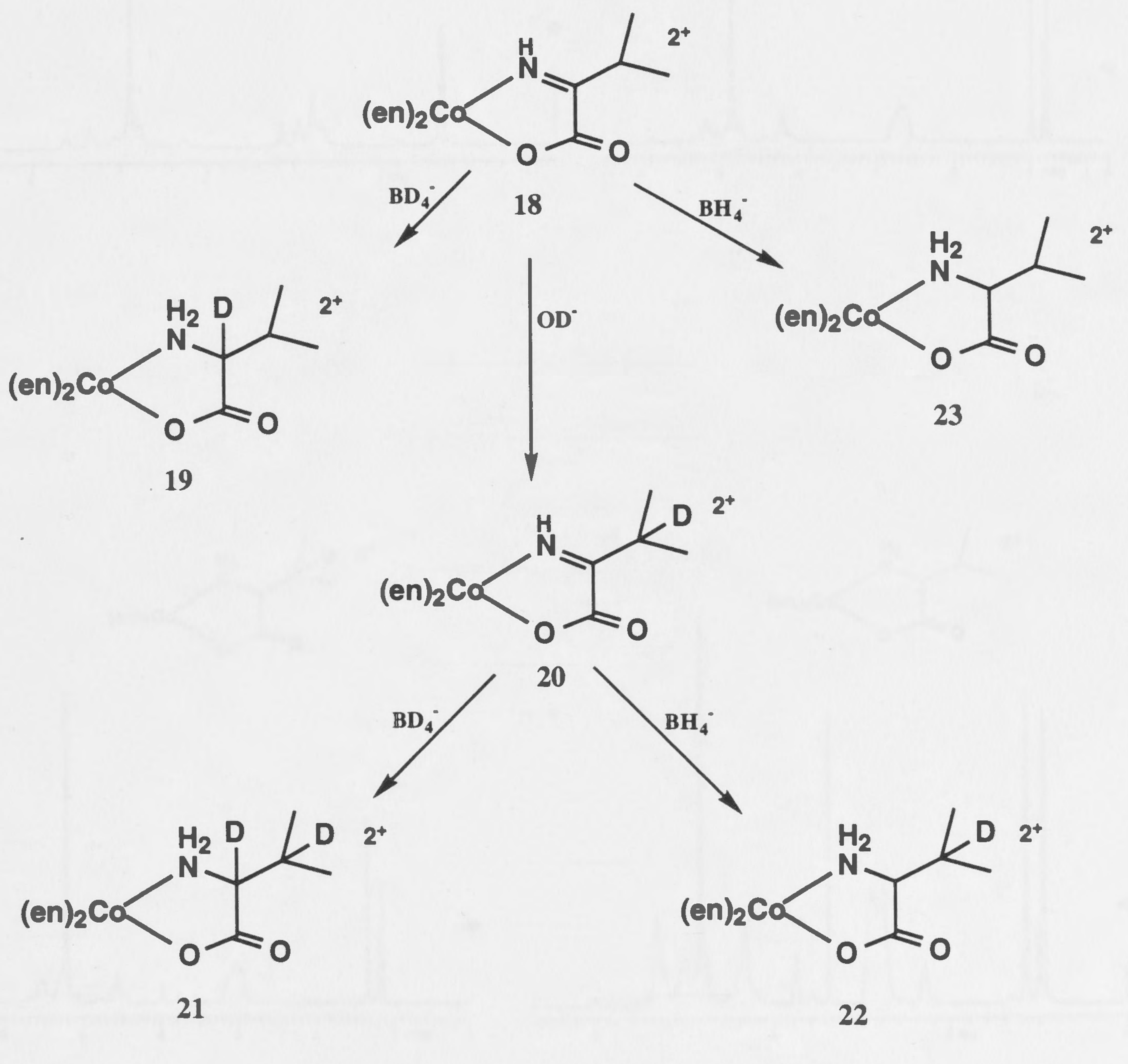


Figure 11: Regioselective deuteriation of valine.

shifted to 1.14 and 0.99/0.94 (depending on the isomer).[14] Signals due to the α-CH group appear at 3.73/3.57 ppm. The proton on the β-carbon of **21** and **22** has been

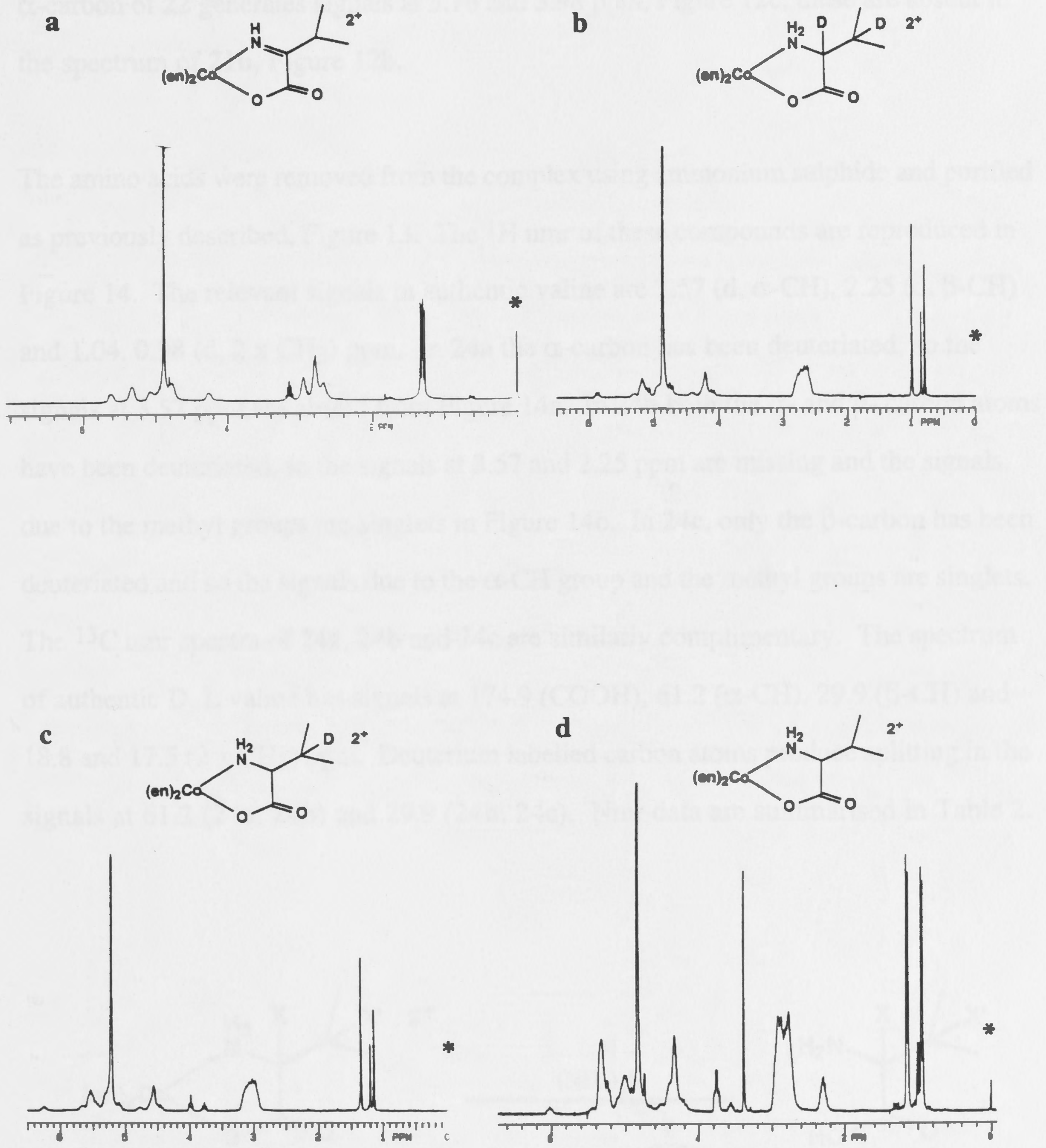
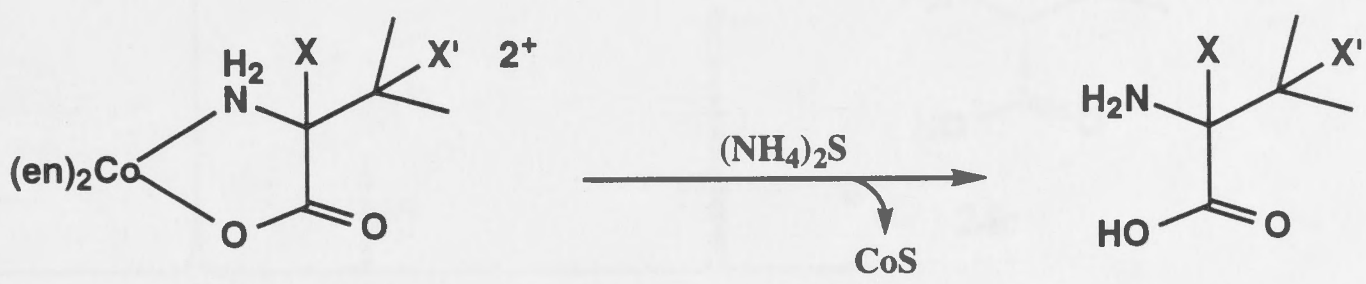



Figure 12: $^1$H nmr spectra (0.1 M DCl, *NaTPS) of the complexes:

**a**: $[(en)_2Co(val\text{-}im)]^{2+}$, **b**: **21** , **c**: **22**, **d**: $[(en)_2Co(val)]^{2+}$.

exchanged with deuterium, so the methyl signals in the corresponding $^1H$ nmr (Figure 12b, 12c) no longer show the coupling with the β-CH. The presence of a proton on the α-carbon of **22** generates signals at 3.76 and 3.98 ppm, Figure 12c; these are absent in the spectrum of **21b**, Figure 12b.

The amino acids were removed from the complex using ammonium sulphide and purified as previously described, Figure 13. The $^1H$ nmr of these compounds are reproduced in Figure 14. The relevant signals in authentic valine are 3.57 (d, α-CH), 2.25 (d, β-CH) and 1.04, 0.98 (d, 2 x $CH_3$) ppm. In **24a** the α-carbon has been deuteriated, so the signals at 3.57 ppm are absent from Figure 14a. In **24b** both the α- and β- carbon atoms have been deuteriated, so the signals at 3.57 and 2.25 ppm are missing and the signals due to the methyl groups are singlets in Figure 14b. In **24c**, only the β-carbon has been deuteriated and so the signals due to the α-CH group and the methyl groups are singlets. The $^{13}C$ nmr spectra of **24a, 24b** and **24c** are similarly complimentary. The spectrum of authentic D, L valine has signals at 174.9 (COOH), 61.2 (α-CH), 29.9 (β-CH) and 18.8 and 17.5 (2 x $CH_3$) ppm. Deuterium labelled carbon atoms produce splitting in the signals at 61.2 (**24a, 24b**) and 29.9 (**24b, 24c**). Nmr data are summarised in Table 2.




**a**: X = D, X' = H
**b**: X = D, X' = D
**c**: X = H, X' = D
**d**: X = H, X' = H

**24**

Figure 13: Isolation of labelled valine.

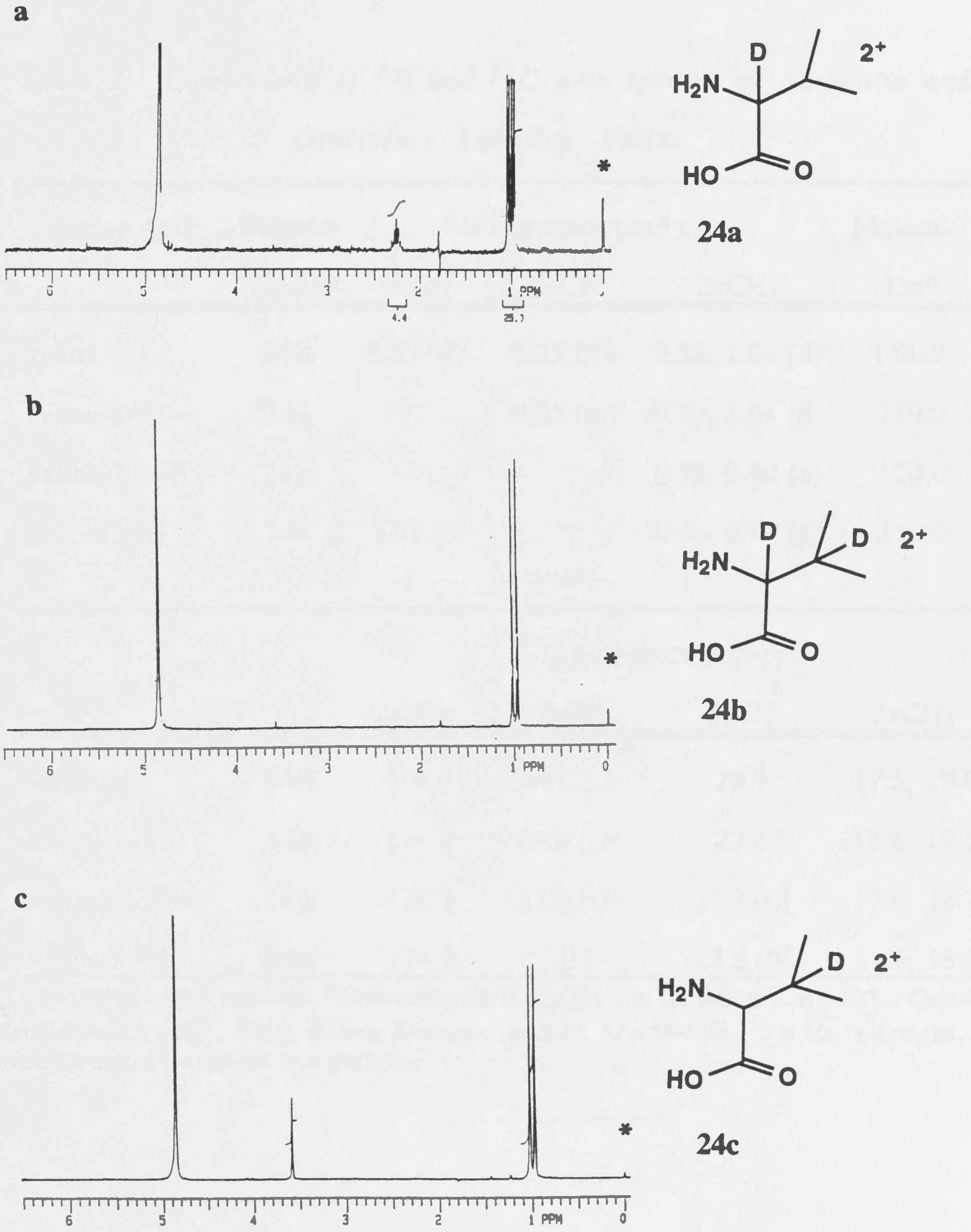



Figure 14: $^{1}H$ nmr spectra ($D_2O$, *NaTPS) of regioselectively labelled valine.

***Table 2: Comparison of $^{1}H$ and $^{13}C$ nmr spectra of Authentic and Deuterium Labelled Valine.***

| Amino Acid | Diagram Number | $^{1}$H nmr spectrum,[a] ppm α-CH | β-CH | $2xCH_3$ | Molecular Ion[b] |
|---|---|---|---|---|---|
| valine | **24d** | 3.57 (d) | 2.25 (m) | 0.98, 1.04 (d) | 118.2[c] |
| valine-2-$^{2}$H | **24a** | - | 2.25 (m) | 0.99, 1.04 (d) | 119.0 |
| valine-2,3-$^{2}$H | **24b** | - | - | 0.98, 0.99 (s) | 120.0 |
| valine-3-$^{2}$H | **24c** | 3.73 (s) | - | 0.98, 0.99 (s) | 119.0 |

| | | $^{13}$C nmr spectrum,[d] ppm COOH | α-CH | β-CH | $2xCH_3$ |
|---|---|---|---|---|---|
| valine | **24d** | 174.9 | 61.2 | 29.9 | 17.5, 18.8 |
| valine-2-$^{2}$H | **24a** | 174.7 | 61.0 (t)[e] | 29.6 | 17.5, 18.6 |
| valine-2,3-$^{2}$H | **24b** | 174.7 | 61.0 (t)[e] | 29.7 (t)[f] | 17.6, 18.7 |
| valine-3-$^{2}$H | **24c** | 174.8 | 61.1 | 29.6 (t)[f] | 17.6, 18.7 |

[a]$D_2O$, NaTPS as internal standard. [b]Electrospray ms ($CH_3CN$), ions are (amino acid + H)$^+$. [c]Calculated (molecular weight + H)$^+$. [d]$D_2O$, dioxane as internal standard. [e]$J$ = 24.4 Hz. [f] $J$ = Hz. s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

*BIOSYNTHETIC STUDIES*

The amino acids **13a**, **13b**, **13c**, **17**, **24a**, **24b**, and **24c** are currently being used in incorporation experiments involving tropical marine sponges collected on Heron Island (see Figure 15) by Dr Mary Garson and coworkers, of the University of Queensland. The methodology of these experiments is similar to that appearing in previous publications[6a] and involves incubating harvested samples of the sponge with each of the amino acid samples for 15 hours before replacing them back on the reef and collecting them for analysis 14 days later. The metabolites of interest (**1**) will be extracted and analysed using nmr and/or gc-ms.

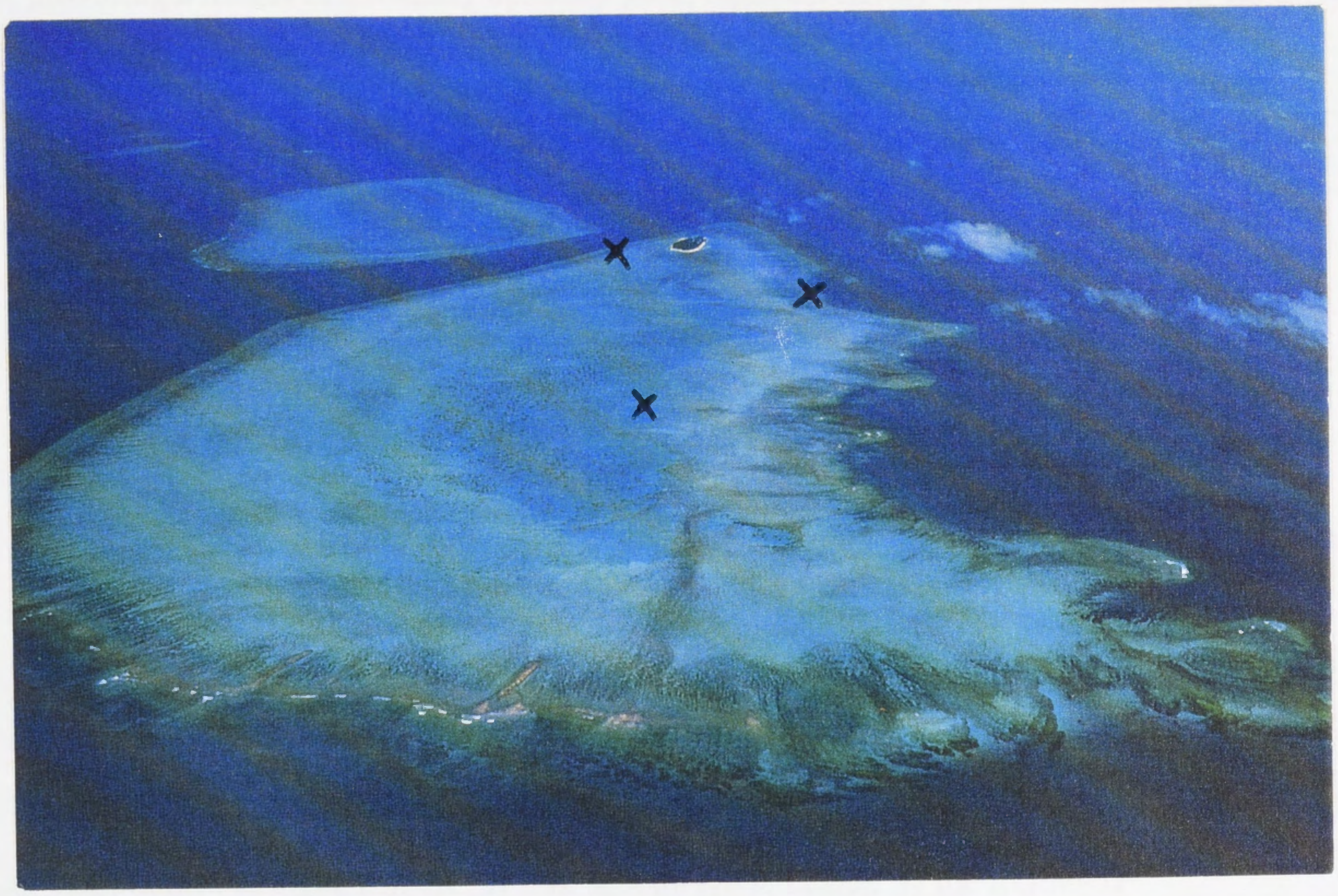


Figure 15: Sites (marked with crosses) around Heron Island from which specimens of marine sponge have been sampled.[16]

### *CONCLUDING REMARKS*

Tetraamine Co(III) complexes of α-imino acids have been used to synthesise a range of deuterium labelled amino acids. The metal ion plays an important role in the reactions. Firstly, it protects the amine group and gives the carboxyl group some ester-like character. Secondly, it activates the imine-C towards nucleophillic attack by $BH_4^-$ or $BD_4^-$, resulting in an amino acid having either hydrogen or deuterium on the α-carbon. Thirdly, it activates the protons on the β-carbon, allowing them to be exchanged readily in basic solution and provide another means of introducing deuterium label(s) into the amino acid. Finally, isomers of the Co(III) complexes may be separated readily,[15] providing a means of obtaining optically pure, regioselectively deuteriated, amino acids.

## Experimental

### *INSTRUMENTS, REAGENTS AND ANALYSES*

$^1H$ and $^{13}C$ nuclear magnetic resonance spectra of the complexes dissolved in $D_2O$ or 0.1M DCl were acquired using a Varian Instruments Gemini 300 NMR spectrometer. Chemical shifts in $^1H$ nmr spectra are reported relative to sodium trimethylsilylpropanesulfonate (NaTPS), 0.00 ppm. Chemical shifts in $^{13}C$ nmr spectra were established relative to dioxane, 67.4 ppm.

Multiplicities of signals in the $^1H$ nmr spectra are indicated by the following abbreviations: singlet (s), doublet (d), triplet (t), quintet (q), multiplet (m), broad (br), AB spin system (AB). Most solvents and basic chemicals used for syntheses were analytical reagent grade. Ion exchange chromatography was performed with analytical grade Dowex 50Wx2 ($H^+$ form, 200 - 400 mesh, Bio-Rad) or SP Sephadex C25 ($Na^+$ form, Pharmacia) cation exchange resins. Complexes present in the collected eluents were recovered by evaporation under water pump vacuum (~ 20 τ) on a Büchi rotary evaporator, with a water bath temperature of less than 40 °C.

Microanalyses were performed by the ANU Microanalytical Service. Electrospray mass spectroscopy was performed by Dr Margaret Shiels and associates of the Department of Chemistry, University of Wollongong, NSW. Biosynthetic studies of the incorporation of the deuteriated amino acids into metabolites by the tropical marine sponge *Dysidea herbacea* were performed by Dr Mary Garson and associates of the Department of Chemistry, University of Queensland, Qld.

## *SYNTHESES*

### *Synthesis of Precursors*

The complex $[(en)_2Co(NHCH(CH_3)_2COO)](ClO_4)_2$ was prepared by a previously published method.[13]

*Synthesis of* $[(en)_2Co(4\text{-}aza\text{-}leu)]Cl_2$ *and Isolation of 4-azaleucine* **17**

The complex $[(en)_2Co(NH_2CH(CH_2N(CH_3)_2)(COO)]Cl_2$ was prepared and 4-azaleucine isolated by a previously established method,[11] from the corresponding Vilsmeier-Haack complex.[12]

*Synthesis of* $[(NH_3)_5Co(OCOCOCH_2CH(CH_3)_2)](ClO_4)_2$ **4**

$[(NH_3)_5Co(OH_2)](ClO_4)_3$ (5.00 g) was stirred into a solution of 1 $cm^3$ of 4 M $HClO_4$ and 9 $cm^3$ $H_2O$. Sodium 4-methyl-α-oxopentanoate (6.08 g) was added to this mixture and the resulting suspension was stirred and heated to 45 °C. The solution and suspension turned red within 30 minutes and were heated for a further 3 hours. The red precipitate was collected by vacuum filtration, and then dissolved in hot water whilst still in the filter funnel. The hot, red solution was cooled on ice, producing dark scarlet crystals. These were collected and washed with a minimum volume of cold water, ethanol and then ether before drying under vacuum overnight (3.90 g, 76%). Analysis calculated for $[CoC_6H_{24}N_5O_{11}Cl_2]$: Co, 12.48; C, 15.26; H, 5.12; N, 14.83; Cl, 15.02. Found: Co, 12.6; C, 15.3; H, 5.3; N, 15.3; Cl, 14.9. $^1H$ nmr (0.1M DCl): $\delta$ 4.1, 3.5 (b, 12H, 4 x *cis* $NH_3$), 3.5 (b, 3H, *trans* $NH_3$), 2.73 (d, 2H, $\beta$-$CH_2$), 2.19 (m, 1H, $\gamma$-CH), 0.99, 0.98 (d, 6H, 2 x $CH_3$). $^{13}C$ nmr (0.1M DCl): $\delta$ 188.2 (Co-O-C=O), 174.0 (C=O), 44.7 ($\gamma$-$CH_2$), 26.5 ($\delta$-CH), 22.7 (2 x $CH_3$).

*Synthesis of $[(NH_3)_4Co(NHC(CH_2CH(CH_3)_2)COO)]Cl_2$ 7*

$[(NH_3)_5Co(OCOCOCH_2CH(CH_3)_2)](ClO_4)_2$ (3.6 g) was dissolved in a solution of NaOH (0.08 M, 95 $cm^3$). The mixture was stirred for 30 seconds at ~ 25 $^oC$ before quenching the reaction by adjusting the pH of the solution to 4 with 3 M acetic acid. After dilution to 500 $cm^3$, the product was purified by ion exchange chromatography on Dowex (2.5 x 15 cm). The adsorbed complexes were washed with water (500 $cm^3$) and with 0.5 M HCl (500 $cm^3$) before being eluted with 2 M HCl . The eluent containing the orange product was reduced to dryness by rotary evaporation and the residual solid dissolved in a minimum volume of water, filtered and $HClO_4$ (70% solution) added until the first sign of precipitation. The solution was refrigerated overnight to complete precipitation. The orange precipitate was collected by vacuum filtration and washed with ethanol and diethyl ether (2.1 g, 59%). Analysis calculated for $[CoC_6H_{22}N_5O_{10}Cl_2]$: Co, 12.98; C, 15.87; H, 4.88; N, 15.42; Cl, 15.61. Found: Co, 12.7; C, 15.6; H, 4.9; N, 15.2; Cl, 14.5. $^1H$ nmr (0.1M DCl): $\delta$ 4.1 (b, 3H, $NH_3$), 3.6 (b, 6H, 2 x $NH_3$), 3.3 (b, 3H, $NH_3$), 2.95 (d, 2H, $\beta$-$CH_2$), 2.13 (m, 1H, $\gamma$-CH), 0.99, 0.98 (d, 6H, 2 x

$CH_3$). $^{13}C$ nmr (0.1M DCl): δ 187.6 (Co-O-Co=O), 173.0 (C=N), 42.3 (β-$CH_2$), 26.2 (γ-CH), 21.5 ($CH_3$).

*Synthesis of $[(NH_3)_4Co(NHC(\mathbf{CD_2}CH(CH_3)_2)COO)]^{2+}$* ***9***

The undeuteriated complex, $[(NH_3)_4Co(NHC(CH_2CH(CH_3)_2)COO)]Cl_2$ (0.50 g), was dissolved in 5 $cm^3$ of a carbonate/deuterobicarbonate buffer ( $[CO_3^{2-}]$ = $[DCO_3^-]$ = 0.25 M, pH = 10.0). The resulting deep orange solution was stirred at room temperature for 10 hours. The exchange of the protons on the β carbon (loss of doublet at 2.72 ppm) was monitored by $^1H$ nmr spectroscopy. This solution was used in the preparation of the deuteriated amino acids which follow. $^1H$ nmr ($CO_3^{2-}$/$DCO_3^-$ buffer): δ 2.18 (m, 1H, CH), 0.99, 0.98 (d, 6H, 2 x $CH_3$).

*Synthesis of $[(en)_2Co(NHC(\mathbf{CD}(CH_3)_2)COO)]Cl_2$* ***20***

The undeuteriated complex, $[(en)_2Co(NHC(CH(CH_3)_2)COO)]Cl_2$ (1.00 g), was dissolved in 20 $cm^3$ of a carbonate/deuterobicarbonate buffer ( $[CO_3^{2-}]$ = $[DCO_3^-]$ = 0.5 M, pH = 10.2). The resulting solution was stirred at room temperature and the exchange of the proton on the β-carbon monitored by $^1H$ nmr spectroscopy. Complete deuteration at this position required about 60 hours, after which time the reaction was quenched by the addition of 3 M acetic acid until the solution had a pH of 3. The complex was adsorbed on a small bed of Dowex resin (50Wx2, $H^+$ form, 2.5 x 5 cm), washed with water and then with 0.5 M HCl before being eluted as a single orange band with 2 M HCl. After rotary evaporation to dryness, the complex was crystallised from dilute acid solution by the addition of ethanol. The product obtained was dried over silica and under vacuum overnight to yield an orange powder (0.95 g, 95%). Analysis calculated for $[CoC_9H_{23}N_5O_2Cl_2D]$: C, 29.60; H+D, 6.90; N, 19.18. Found: C, 29.4; H+D, 7.2; N, 19.3. $^1H$ nmr (0.1 M DCl): δ 4.2 - 5.8 (b, 8H, en-$NH_2$), 2.5 - 3.5 (b, 8H, en-$CH_2$), 0.97, 0.99 (s, 6H, 2 x $CH_3$).

***Synthesis of Deuteriated Amino Acids***

*Synthesis of $[(NH_3)_4Co(NH_2CD(CH_2CH(CH_3)_2)COO)]^{2+}$* ***8***

$[(NH_3)_4Co(NHC(CH_2CH(CH_3)_2)COO)]Cl_2$ (0.25 g) was dissolved in 10 $cm^3$ of a carbonate/bicarbonate buffer solution ($[CO_3^{2-}] = [HCO_3^-] = 0.5$ M). $NaBD_4$ (0.08 g) was added and the resulting mixture stirred vigorously at room temperature for 60 seconds before quickly passing it through Dowex (50Wx2, $Na^+$ form, 2.5 x 5.0 cm) under suction. The adsorbed material was washed with water whilst under suction. Co(II) was eluted with 0.5 M HCl as the column recovered from suction. The remaining orange material was eluted as a single fraction with a small volume of 2 M HCl. The acid was removed by rotary evaporation to leave an orange solid. $^1H$ nmr (0.1M DCl): $\delta$ 6.25, 5.20 (ABXq, 2H, $NH_2$), 3.9 (b, 3H, $NH_3$), 3.7 (b, 6H, 2 x $NH_3$), 3.1 (b, 3H, $NH_3$), 1.95 (m, 1H, $\gamma$-CH), 1.88 (ABXq, 2H, $\beta$-$CH_2$), 0.98, 0.99 (d, 2H, 2 x $CH_3$). The deuteriated amino acid was removed from this complex without further purification.

*Synthesis of $[(NH_3)_4Co(NH_2CD(CD_2CH(CH_3)_2)COO))^{2+}$* ***10***

A portion of $NaBD_4$ (0.08 g) was added to 2.5 $cm^3$ of the solution containing $[(NH_3)_4Co(NHC(\mathbf{CD_2}CH(CH_3)_2)COO])^{2+}$ and the resulting mixture stirred vigorously at room temperature for 40 seconds before quickly passing it through Dowex (50Wx2, $Na^+$ form, 2.5 x 5.0 cm) under suction. The adsorbed material was washed with water whilst under suction. Co(II) was eluted with 0.5 M HCl as the column recovered from suction. The remaining orange material was eluted as a single fraction with a small volume of 2 M HCl. The acid was removed by rotary evaporation to leave an orange solid. $^1H$ nmr (0.1 M DCl): $\delta$ 6.15, 5.20 (AXq, 2H, $NH_2$), 4.0 (b, 3H, $NH_3$), 3.61 (b, 6H, 2 x $NH_3$), 3.1 (b, 3H, $NH_3$), 1.95 (m, 1H, $\gamma$-CH), 0.99, 0.98 (d, 2H, 2 x $CH_3$). The deuteriated amino acid was removed from this complex without further purification.

*Synthesis of* $[(NH_3)_4Co(NH_2CH(CD_2CHCH_3)_2)COO)]^{2+}$ ***11***

A portion of $NaBH_4$ (0.08 g) was added to 2.5 $cm^3$ of the solution containing $[(NH_3)_4Co(NHC(CD_2CH(CH_3)_2)COO)]^{2+}$ and the resulting mixture stirred vigorously at room temperature for 40 seconds before quickly passing it through Dowex ( 50Wx2, $Na^+$ form, 2.5 x 5.0 cm) under suction. The adsorbed material was washed with water whilst under suction before traces of Co(II) were eluted with 0.5 M HCl as the column recovered from suction. The remaining orange material was eluted as a single fraction with a small volume of 2 M HCl. The acid was removed by rotary evaporation to leave an orange solid. $^1H$ nmr (0.1 M DCl): δ 6.22, 5.20 (AXq, 2H, $NH_2$),4.0 (b, 3H, $NH_3$), 3.72 (s, 1H, α-CH), 3.7 (b, 6H, 2 x $NH_3$), 3.1 (b, 3H, $NH_3$), 1.95 (m, 1H, γ-CH), 0.98, 0.99 (d, 2H, 2 x $CH_3$). The deuteriated amino acid was removed from this complex without further purification.

*Synthesis of* $[(en)_2Co(NH_2CD(CH(CH_3)_2)COO)]Cl_2$ ***19***

$[(en)_2Co(NHC(CH(CH_3)_2)COO)]Cl_2$ (0.50 g), was dissolved in 10 $cm^3$ of a sodium carbonate/bicarbonate buffer ( $[CO_3^{2-}] = [HCO_3^-] = 0.5$ M, pH = 10.3 ). $NaBD_4$ (0.16 g) was added and the resulting mixture stirred vigorously at room temperature for 60 seconds before quickly passing it through Dowex (50Wx2, $Na^+$ form, 2.5 x 5.0 cm) under suction. The adsorbed material was washed with water whilst under suction. Some Co(II) was eluted with 0.5 M HCl as the column recovered from suction. The remaining orange material was eluted as a single fraction with a small volume of 4 M HCl. The acid was removed by rotary evaporation to leave an orange solid which proved by nmr to be a virtually pure sample of the desired complex, (0.40 g, 99%). The deuteriated amino acid was removed from this complex without further purification. $^1H$ nmr (0.1 M DCl): δ 5.88, 5.10 (AXq, 2H, $NH_2$), 4.0 - 5.3 (b, 8H, en-$NH_2$), 2.5 - 2.9 (b, en-$CH_2$), 2.35 (m, 1H, β-CH), 0.88, 0.91 (d, 6H, 2 x $CH_3$).

*Synthesis of [(en)$_2$Co(NH$_2$CD(CD(CH$_3$)$_2$)COO))$^{2+}$* ***21***

[(en)$_2$Co(NHC(CH(CH$_3$)$_2$)COO)](ClO$_4$)$_2$ (0.50 g), was dissolved in 9.5 cm$^3$ of the carbonate/deuterobicarbonate buffer described above. The resulting solution was stirred at room temperature for 60 hours. $^1$H nmr confirmed the complete exchange of the proton on the β-carbon and the solution was diluted to 10 cm$^3$ with D$_2$O. A portion of NaBD$_4$ (0.14 g) was added and the resulting mixture stirred vigorously at room temperature for 60 seconds before quickly passing it through Dowex (50Wx2, Na$^+$ form, 2.5 x 5.0 cm) under suction. The adsorbed material was washed with water whilst under suction. Traces of Co(II) was eluted with 0.5 M HCl as the column recovered from suction. The remaining orange material was eluted as a single fraction with a small volume of 4 M HCl. The acid was removed by rotary evaporation to leave an orange solid, (0.41g, 99%). The nmr spectra of this material showed it to be a virtually pure sample of the desired complex and the deuteriated amino acid was removed from this complex without further purification. $^1$H nmr (0.1 M DCl): δ 5.88, 5.10 (AXq, 2H, NH$_2$), 4.0 - 5.3 (b, 8H, en-NH$_2$), 2.5 - 2.9 (b, en-CH$_2$), 0.88, 0.91 (s, 6H, 2 x CH$_3$).

*Synthesis of [(en)$_2$Co(NH$_2$CH(CD(CH$_3$)$_2$)COO)]$^{2+}$* ***22***

[(en)$_2$Co(NHC(CH(CH$_3$)$_2$)COO)]Cl$_2$ (0.50 g), was dissolved in 9.5 cm$^3$ of the carbonate/deuterobicarbonate buffer described above. The resulting solution was stirred at room temperature for 60 hours. $^1$H nmr spectroscopy confirmed the complete exchange of the proton on the β carbon and the solution was diluted to 10 cm$^3$ with D$_2$O. A portion of NaBH$_4$ (0.14 g) was added and the resulting mixture stirred vigorously at room temperature for 60 seconds before quickly passing it through Dowex (50Wx2, Na$^+$ form, 2.5 x 5.0 cm) under suction. The adsorbed material was washed with water whilst under suction. A very small quantity of Co(II) was eluted with 0.5 M HCl as the column recovered from suction. The remaining orange material was eluted as a single fraction

HCl. The acid was removed by rotary evaporation to leave an orange solid, (0.40g, 99%) This material was shown to be the desired complex and to be pure by nmr and the deuteriated amino acid was removed from this complex without further purification. $^{1}H$ nmr (0.1M DCl): $\delta$ 5.88, 5.10 (AXq, 2H, $NH_2$), 4.0 - 5.3 (b, 8H, en-$NH_2$), 6.25, 5.65 (AXq, 2H, $NH_2$), 4.4 - 5.6 (b, 8H, en-$NH_2$), 3.76, 3.98 (t, 1H, $\alpha$-CH), 2.5 - 2.9 (b, en-$CH_2$), 0.88, 0.91 (d, 6H, 2 x $CH_3$).

## *Isolation of Deuteriated Amino Acids*

### *Isolation of $NH_2CD(CH_2CH(CH_3)_2)COOH$* ***13a***

The residue containing $[(NH_3)_4Co(NH_2\mathbf{CD}(CH_2CH(CH_3)_2)COO)]Cl_2$ was dissolved in water (10 $cm^3$) and an 8% solution of $(NH_4)_2S$ added dropwise to remove the cobalt. The resulting suspension was left to stand at room temperature for 30 minutes to complete precipitation of CoS before filtering it through Filter Aid. The pH of this solution was adjusted to 1 with 2 M HCl before adsorbing it to a column of Dowex resin (50Wx2, $H^+$ form, 3 x 15 cm). The adsorbed material was washed with water (50 $cm^3$) before eluting with 0.5 M ammonia solution. The eluate was collected in fractions of about 30 $cm^3$; the amino acid was found in fractions 5 and 6. These were combined and taken to dryness by rotary evaporation before being recrystallised twice from $H_2O$/isopropanol to give a white powder which was dried for one week under vacuum and over silica (0.03 g, 41%). Analysis calculated for $[C_6H_{12}\mathbf{D}NO_2]$: C, 54.52; H, 9.15; N, 10.60. Found: C, 54.4; H, 9.13; N, 10.5. $^{1}H$ nmr ($D_2O$): $\delta$ 1.70, (m, 1H, $\gamma$-CH), 1.75 (ABXq, 2H, $\beta$-$CH_2$), 0.97, 0.98 (d, 6H, 2 x $CH_3$). $^{13}C$ nmr ($D_2O$): $\delta$ 187.9 (COOH), 56.8 (t, $J_{C\text{-}D}$ 24.4 Hz, $\alpha$-**CD**), 42.4 ($\beta$-$CH_2$), 24.9 ($\gamma$-CH), 20.9, 21.0 (2 x $CH_3$). Electrospray mass spectrum ($CH_3CN$): 133.0 (calculated for $NH_2\mathbf{CD}(CH_2CH(CH_3)_2)(COOH)$ + H: 132.2), 87.0.

*Isolation of $NH_2CD(CD_2CH(CH_3)_2)COOH$ 13b*

The residue containing $[(NH_3)_4Co(NH_2\mathbf{CD}(\mathbf{CD}_2CH(CH_3)_2)COO)]Cl_2$ was dissolved in water (10 $cm^3$) and an 8% solution of $(NH_4)_2S$ added dropwise to remove the cobalt. The resulting suspension was left to stand at room temperature for 30 minutes to complete precipitation of CoS before filtering it through Filter Aid. The pH of this solution was adjusted to 1 with 2 M HCl before adsorbing it to a column of Dowex resin (50Wx2, $H^+$ form, 3 x 15 cm). The adsorbed material was washed with water (50 $cm^3$) before eluting with 0.5 M ammonia solution. The eluate was collected in fractions of about 30 $cm^3$; the amino acid was found in fractions 5 and 6. These were combined and taken to dryness by rotary evaporation before being recrystallised twice from $H_2O$/isopropanol to give a white powder which was dried for one week under vacuum and over silica (0.03 g, 41%). Analysis calculated for $[C_6H_{10}\mathbf{D}_3NO_2]$: C, 53.70; H, 7.51; N, 10.44. Found: C, 53.9; H, 7.5; N, 10.3. $^1H$ nmr ($D_2O$): $\delta$ 1.70, (m, 1H, $\gamma$-CH), 0.97, 0.98 (d, 6H, 2 x $CH_3$). $^{13}C$ nmr ($D_2O$): $\delta$ 187.9 (COOH), 56.6 (t, $J_{C\text{-}D}$ 24.4 Hz, $\alpha$-**CD**), 42.4 (m, $\beta$-$\mathbf{CD}_2$), 24.5 ($\gamma$-CH), 19.8, 20.9 (2 x $CH_3$). Electrospray mass spectrum ($CH_3CN$): 135.0 (calculated for $NH_2\mathbf{CD}(\mathbf{CD}_2CH(CH_3)_2)(COOH)$ + H: 135.2), 89.0.

*Isolation of $NH_2CH(CD_2CH(CH_3)_2)COOH$ 13c*

The residue containing $[(NH_3)_4Co(NH_2CH(\mathbf{CD}_2CH(CH_3)_2)COO)]Cl_2$ was dissolved in water (10 $cm^3$) and an 8% solution of $(NH_4)_2S$ added dropwise to remove the cobalt. The resulting suspension was left to stand at room temperature for 30 minutes to complete precipitation of CoS before filtering it through Filter Aid. The pH of this solution was adjusted to 1 with 2 M HCl before adsorbing it to a column of Dowex resin (50Wx2, $H^+$ form, 3 x 15 cm). The adsorbed material was washed with water (50 $cm^3$) before eluting with 0.5 M ammonia solution. The eluate was collected in fractions of about 30 $cm^3$; the amino acid was found in fractions 6 and 7. These were combined and taken to dryness by rotary evaporation before being recrystallised twice from $H_2O$/isopropanol and to give a white powder which was dried for one week under vacuum and over silica (0.03 g, 41%). Analysis calculated for [$C_6H_{11}\mathbf{D}_2NO_2$]: C, 54.11; H, 8.32; N, 10.52. Found: C, 53.2; H, 8.5; N, 10.4. $^1$H nmr ($D_2O$): $\delta$ 3.73 (s, 1H, $\alpha$-CH), 1.69 (m, 1H, $\gamma$-CH), 0.97, 0.98 (d, 6H, 2 x $CH_3$). $^{13}$C nmr ($D_2O$): $\delta$ 187.8 (COOH), 56.5 ($\alpha$-CH), 42.3 (m, $\mathbf{CD}_2$), 24.5 ($\gamma$-CH), 19.9, 20.9 (2 x $CH_3$). Electrospray mass spectrum ($CH_3CN$): 134.0 (calculated for $NH_2CH(\mathbf{CD}_2CH(CH_3)_2)(COOH)$ + H: 133.2), 88.0.

*Isolation of $NH_2CD(CH(CH_3)_2)COOH$ **24a***

The residue containing $[(en)_2Co(NH_2\mathbf{CD}(CH(CH_3)_2)COO)]^{2+}$ was dissolved in water (10 cm$^3$) and an 8% solution of $(NH_4)_2S$ added dropwise to remove the cobalt. The resulting suspension was left to stand at room temperature for 30 minutes to complete precipitation of CoS before filtering it through Filter Aid. The pH of this solution was adjusted to 1 with 2 M HCl before adsorbing it to a column of Dowex resin (50Wx2, $H^+$ form, 3 x 16 cm). The adsorbed material was washed with water (50 cm$^3$) before eluting with 0.5 M ammonia solution. The eluate was collected in fractions of about 25 cm$^3$; the amino acid was found in fractions 8, 9 and 10. These were combined and taken to dryness by rotary evaporation before being recrystallised twice from $H_2O$/isopropanol to give a white powder which was dried for one week under vacuum and over silica (0.06 g, 50%). Analysis calculated for [$C_5H_{10}DNO_2$]: C, 50.83; H, 8.53; N, 11.85. Found: C, 50.5; H, 8.7; N, 11.6. $^1$H nmr ($D_2O$): $\delta$ 2.25 (m, 1H, $\beta$-CH), 0.99, 1.04 (d, 6H, 2 x $CH_3$). $^{13}$C nmr ($D_2O$): $\delta$ 174.7 (COOH), 61.0 (t, $J_{C\text{-}D}$ 24.4 Hz, $\alpha$-CH), 29.6 ($\beta$-CH), 17.5, 18.6 (2 x $CH_3$). Electrospray mass spectrum ($CH_3CN$): 119.0 (calculated for $NH_2\mathbf{CD}(CH(CH_3)_2)COOH$ + H: 118.2), 72.9.

*Isolation of $NH_2CD(CD(CH_3)_2)COOH$ **24b***

The residue containing $[(en)_2Co(NH_2\mathbf{CD}(\mathbf{CD}(CH_3)_2)COO)]^{2+}$ was dissolved in water (10 $cm^3$) and an 8% solution of $(NH_4)_2S$ added dropwise to remove the cobalt. The resulting suspension was left to stand at room temperature for 30 minutes to complete precipitation of CoS before filtering it through Filter Aid to obtain a clear, colourless solution. The pH of this solution was adjusted to 1 with 2 M HCl before adsorbing it to a column of Dowex resin (50Wx2, $H^+$ form, 3 x 17 cm). The adsorbed material was washed with water (50 $cm^3$) before eluting with 0.5M ammonia solution. The eluate was collected in fractions of about 25 $cm^3$; the amino acid was found in fractions 10, 11 and 12. These were combined and taken to dryness by rotary evaporation before being recrystallised twice from $H_2O$/isopropanol to give a white powder that was dried overnight under vacuum and over silica (0.06 g, 50%). Analysis calculated for $[C_5H_{19}\mathbf{D}_2NO_2]$: C, 50.40; H, 7.61; N, 11.75. Found: C, 50.2; H, 7.8; N, 11.7. $^1H$ nmr ($D_2O$): $\delta$ 0.97, 0.98 (s, 2 x $CH_3$). $^{13}C$ nmr ($D_2O$): $\delta$ 174.7 (COOH), 61.0 (t, $J_{C\text{-}D}$ 25.0 Hz, $\alpha$-CH), 29.7 (t, $J_{C\text{-}D}$ 24.4 Hz, $\beta$-CH), 17.6, 18.7 (2 x $CH_3$). Electrospray mass spectrum ($CH_3CN$): 120.0 (calculated for $NH_2\mathbf{CD}(\mathbf{CD}(CH_3)_2)COOH$ + H: 119.2), 73.9.

*Isolation of $NH_2CH(CD(CH_3)_2)COOH$ **24c***

The residue containing $[(en)_2Co(NH_2CH(\mathbf{CD}(CH_3)_2)COO)]^{2+}$ was dissolved in water (10 $cm^3$) and an 8% solution of $(NH_4)_2S$ added dropwise to remove the cobalt. The resulting suspension was left to stand at room temperature for 30 minutes to complete precipitation of CoS before filtering it through Filter Aid the resulting solution was not quite colourless so a little more $(NH_4)_2S$ was added and the solution filtered twice more through Filter Aid before a clear colourless solution was obtained. The pH of this solution was adjusted to 1 with 2 M HCl before adsorbing it to a column of Dowex resin (50Wx2, $H^+$ form, 3 x 16 cm). The adsorbed material was washed with water (50 $cm^3$) before eluting with 0.5 M ammonia solution. The eluate was collected in fractions of about 25 $cm^3$; the amino acid was found in fractions 8, 9 and 10. These were combined and taken to dryness by rotary evaporation before being recrystallised twice from $H_2O$/isopropanol to give a white powder which was dried for a week under vacuum and over silica (0.06 g, 50%). Analysis calculated for [$C_5H_{10}DNO_2$]: C, 50.83; H, 8.53; N, 11.85. Found: C, 50.5; H, 8.9; N, 11.6. $^1$H nmr ($D_2O$): $\delta$ 3.58 (s, 1H, $\alpha$-CH), 0.97, 0.99 (s, 6H, 2 x $CH_3$). $^{13}$C nmr ($D_2O$): $\delta$ 174.8 (COOH), 61.1 ($\alpha$-CH), 29.6 (t, $J_{C\text{-}D}$ 25.0 Hz, $\beta$-CH), 21.8, 17.6, 18.7 (2 x $CH_3$). Electrospray mass spectrum ($CH_3CN$): 119.0 (calculated for $NH_2CD(\mathbf{CD}(CH_3)_2)COOH$ + H: 118.2), 72.9.

**BIOSYNTHETIC STUDIES**

Firstly, 10 mg of each amino acid sample (**13a-c**, **17**, **24a-c**) is incubated with 5 - 10 g of intact sponge tissue maintained under ambient temperature and light in 200 cm$^3$ of aerated sea water for 15 hours (6 pm - 9 am). The sponge samples are then placed underwater at ~ 14 m for 14 days. After this time they are collected and processed to extract the metabolites of interest.

Secondly, 1.0 mg of an amino acid sample is incubated with a cell homogenate from 30 g of the blended sponge. The metabolites are extracted in the same manner as in the first experiments.

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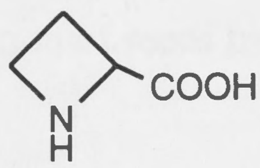
16 Photograph from Nucolorvue Productions Pty. Ltd., Australia.

# CHAPTER 4

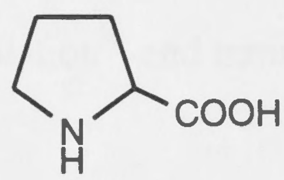
## Self Condensation of $[N_4Co(ala\text{-}im)]^{2+}$

## Introduction

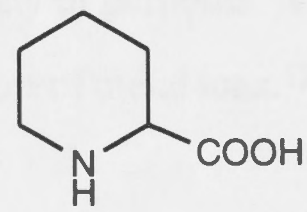
Heterocyclic amino acids are interesting synthetic targets because of their diverse biological functions. Some of the most thoroughly studied include derivatives of azetidine-2-carboxylic acid, **1**, proline , **2**, and pipecolic acid, **3**. Individually, members of these families have innumerable roles in biological systems and they are also incorporated into larger structures such as proteins and antibiotics.



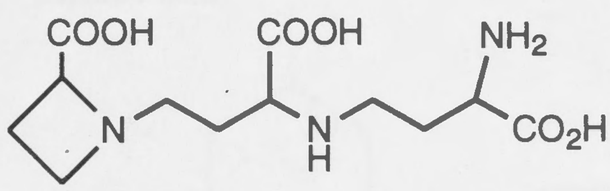
**1**
azetidine-2-carboxylic acid
*Beta vulgaris* (sugar beet)[1]
*Delonia regia*[2]



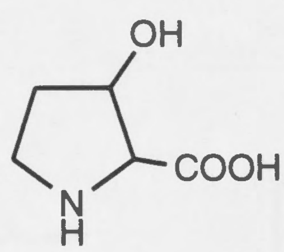
**2**
proline
ubiquitous,
proteins[3]



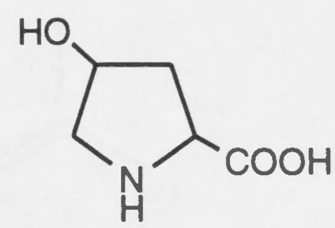
**3**
pipecolic acid
*Phaseolus vulgaris*[4]
(green beans)
*Beta vulgaris*[4]



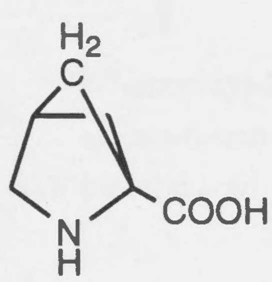
**4**
nicotinimine
*Nicotiana tabacum*[5]



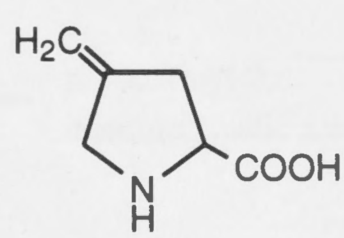
**5**
*trans*-3-hydroxyproline
*Delonix regia*[2]



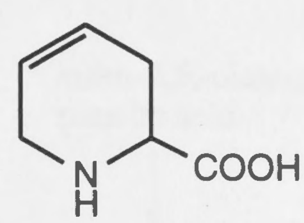
**6**
*trans*-4-hydroxyproline
fibrous connective tissue[3]



**7**
2,4-methanoproline
*Ateleia herbert smithii*[6]



**8**
4-methylene proline
*Eriobotrya japonica*[7]
(loquat)



**9**
Baikiain
*Baikiaea plurijuga*[8]
*Caelspininia tinctoria*[8]

Figure 1: Naturally occurring heterocyclic amino acids.[11]

Collagen is an example of such macromolecules and proline, **2**, and *trans*-4-hydroxyproline, **6**, constitute about 21% of the protein.[3] These amino acids are also present, in smaller quantities, in the related elastins.

In addition to proteins, many heterocyclic amino acids are incorporated into the structures of secondary metabolites. For example *cis*- and *trans*- 3-hydroxy-L-proline are both present in the antibiotic telomycin[9], first isolated from an unidentified *Streptomyces* species.[10] Derivatives of **1 - 3** also act as intermediates in the syntheses of straight chain amino acids. The conversion of aspartic acid to lysine, Figure 2, in higher plants is an example.[11] Finally, non-protein amino acids are employed by biological systems for a wide variety of purposes. These include protection of seeds from predation[6], and transport of metal ions.[12]

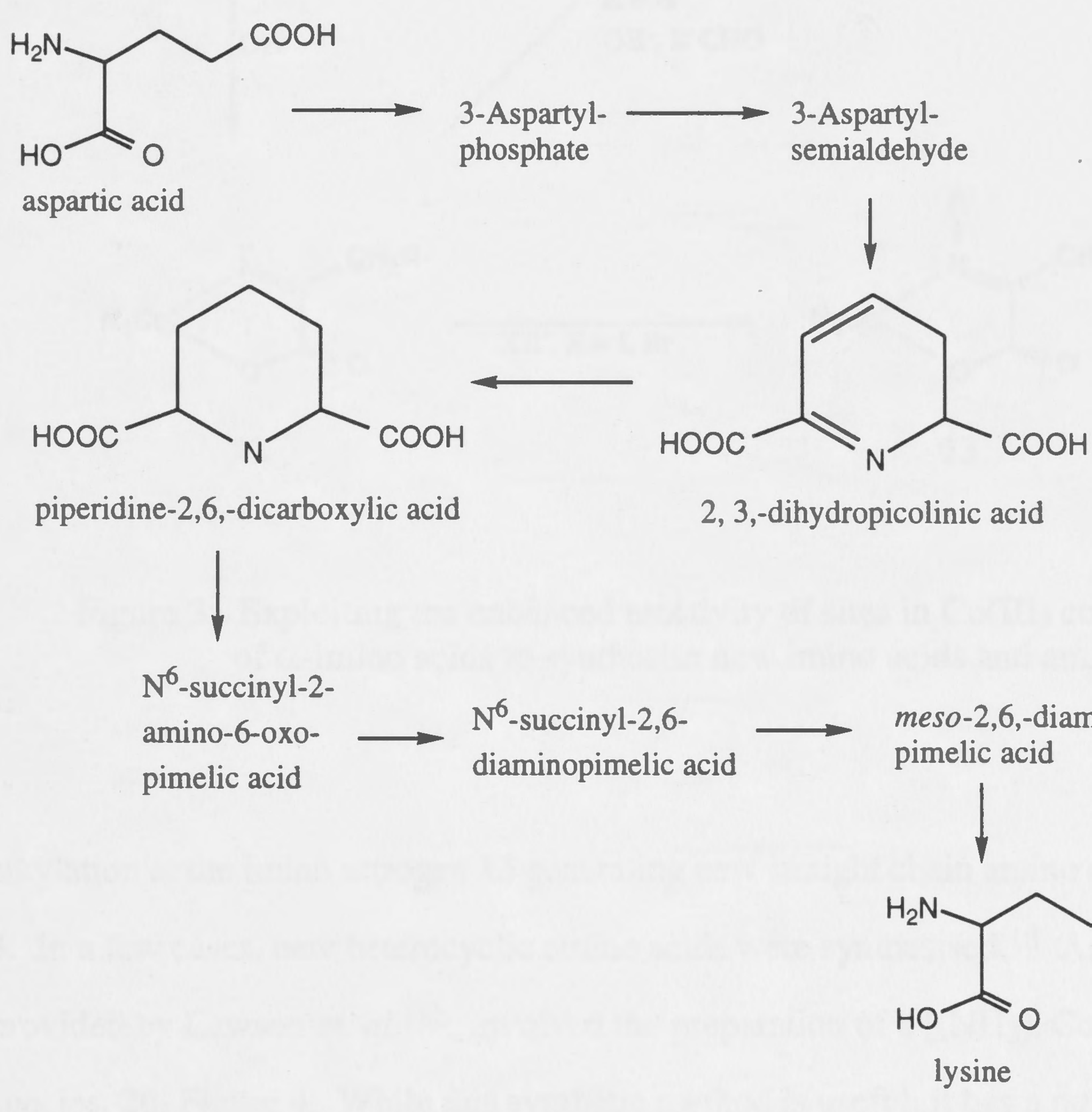



Figure 2: The biosynthesis of lysine from aspartic acid requires heterocyclic amino acids as intermediates.[11]

The use of Co(III) coordination complexes has been a successful means of synthesising both naturally occurring, previously characterised amino acids and new amino acids.[13-18] In most instances, these syntheses involve modification of the side chain **11** or

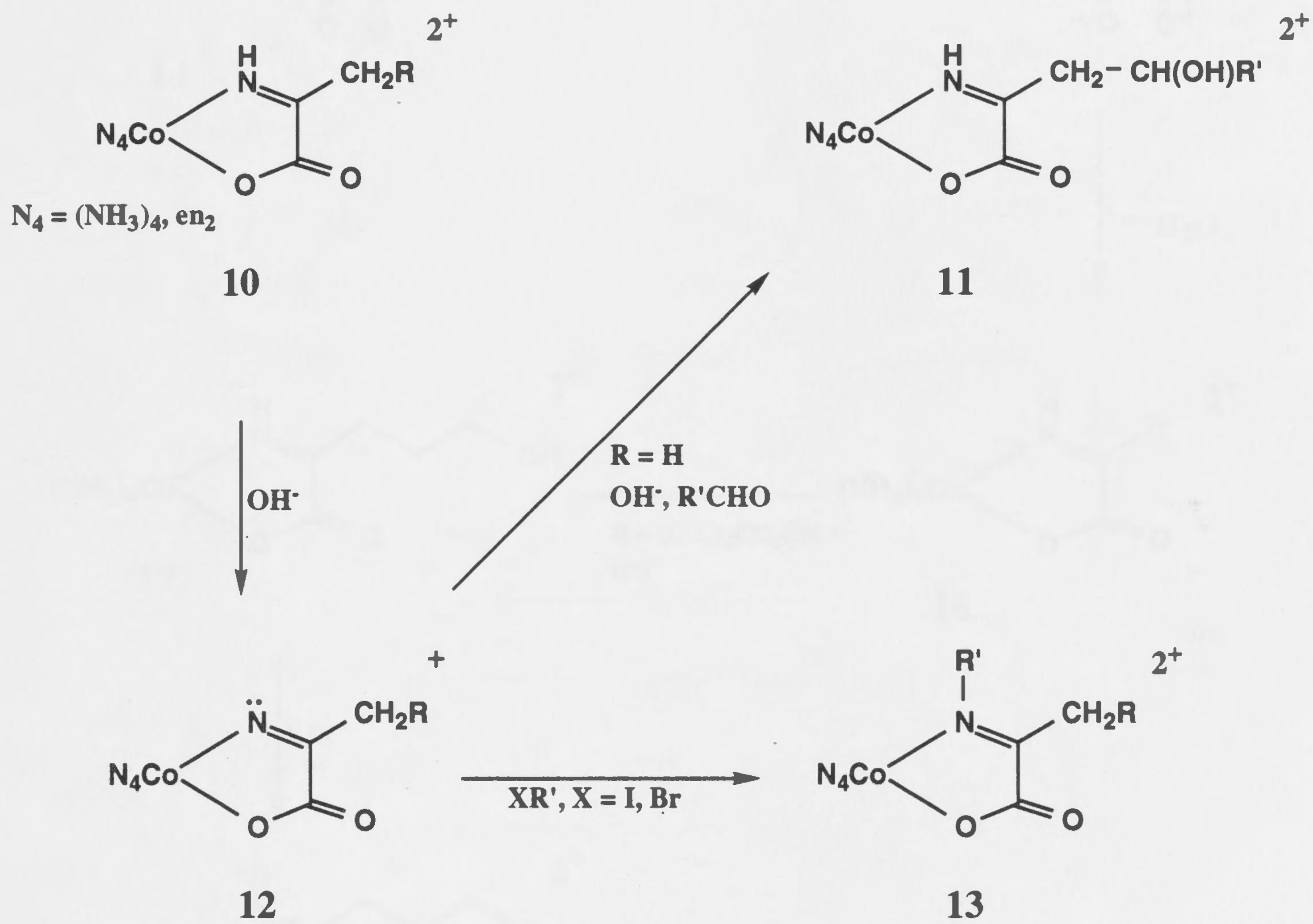


Figure 3: Exploiting the enhanced reactivity of sites in Co(III) complexes of α-imino acids to synthesise new imino acids and amino acids.

alkylation at the imino nitrogen **13** generating new straight chain amino acids, Figure 3. In a few cases, new heterocyclic amino acids were synthesised.[18] An example, provided by Lawson *et. al.*[18b], involved the preparation of a $[(NH_3)_4Co(\text{prolinato})]^{2+}$ species, **20**, Figure 4. While this synthetic method is useful, it has a number of disadvantages. Firstly, the utility of the Schiff base condensation relies on the range of α-ketoacids available. Secondly, tetraammine complexes are less stable under basic conditions than other Co(III) tetraamine complexes. Lastly, the complex cannot induce any stereospecificity into the reaction. Given these factors a new method of synthesising imino acids such as **1 - 3** was proposed.

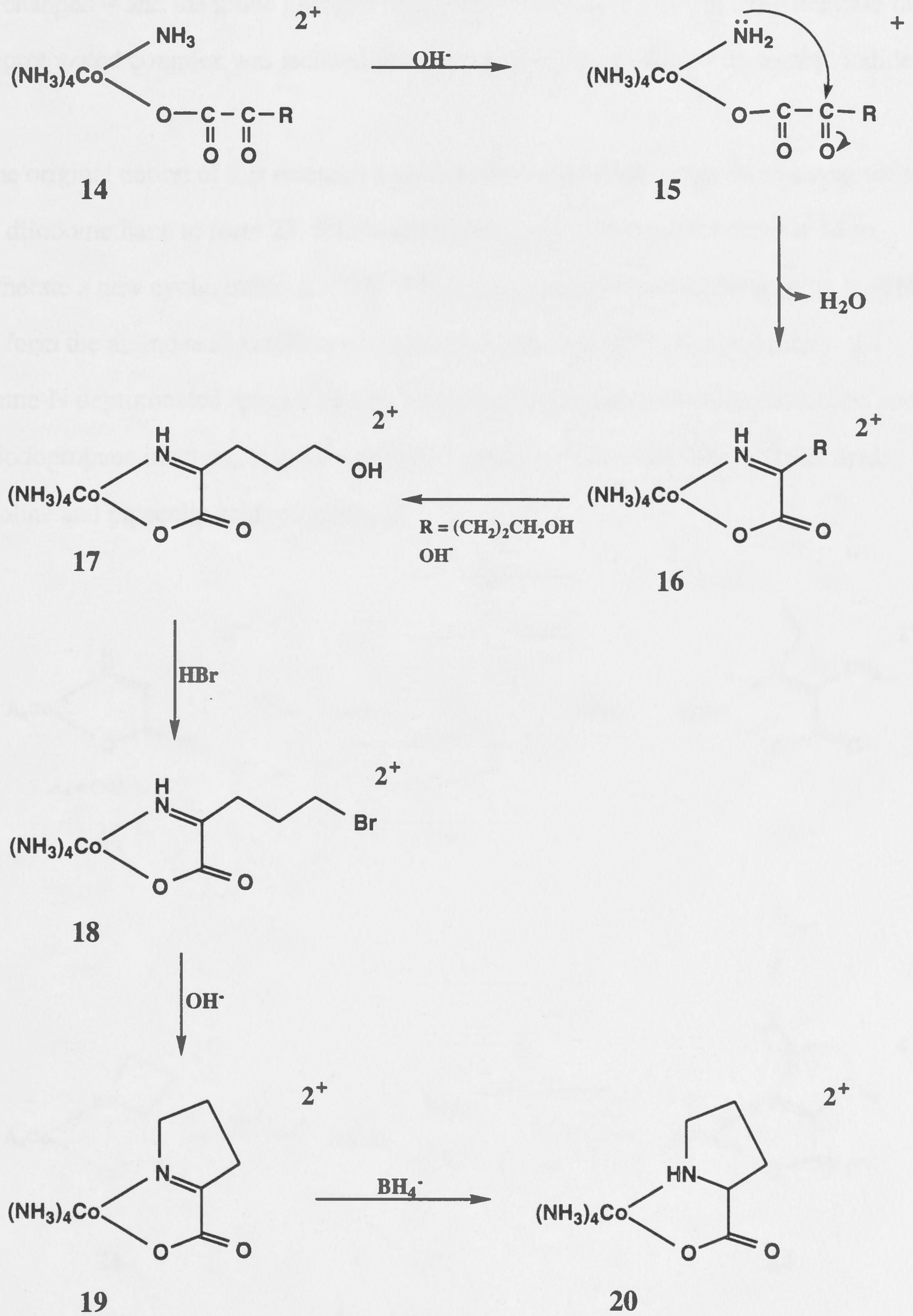


Figure 4: Synthesis of proline from $[(NH_3)_4Co(ala\text{-}im)]^{2+}$.

Early papers describing the synthesis and properties of a chelated imino acid (**10**, $N_4 = (NH_3)_4$, R = H), noted that under basic conditions protons of the methyl group exchanged[15] and the imine nitrogen was readily deprotonated.[13] In the latter case the deprotonated complex was isolated and subsequently methylated with methyl iodide[14].

The original notion of this research involved alkylation at the imine-N by a reagent such as diiodomethane to form **23**, followed by attack of the adjacent carbanion **24** to generate a new cyclic imino acid **25**. The imine would be readily reduced by $NaBH_4$ to form the amino acid azetidine-2-carboxylate **26** (Figure 5). Consequently, the imine-N deprotonated species **22** was reacted with diiodomethane, diiodoethane and diiodopropane in attempts to synthesise complexes of azetidine-2-carboxylic acid, proline and pipecolic acid respectively.

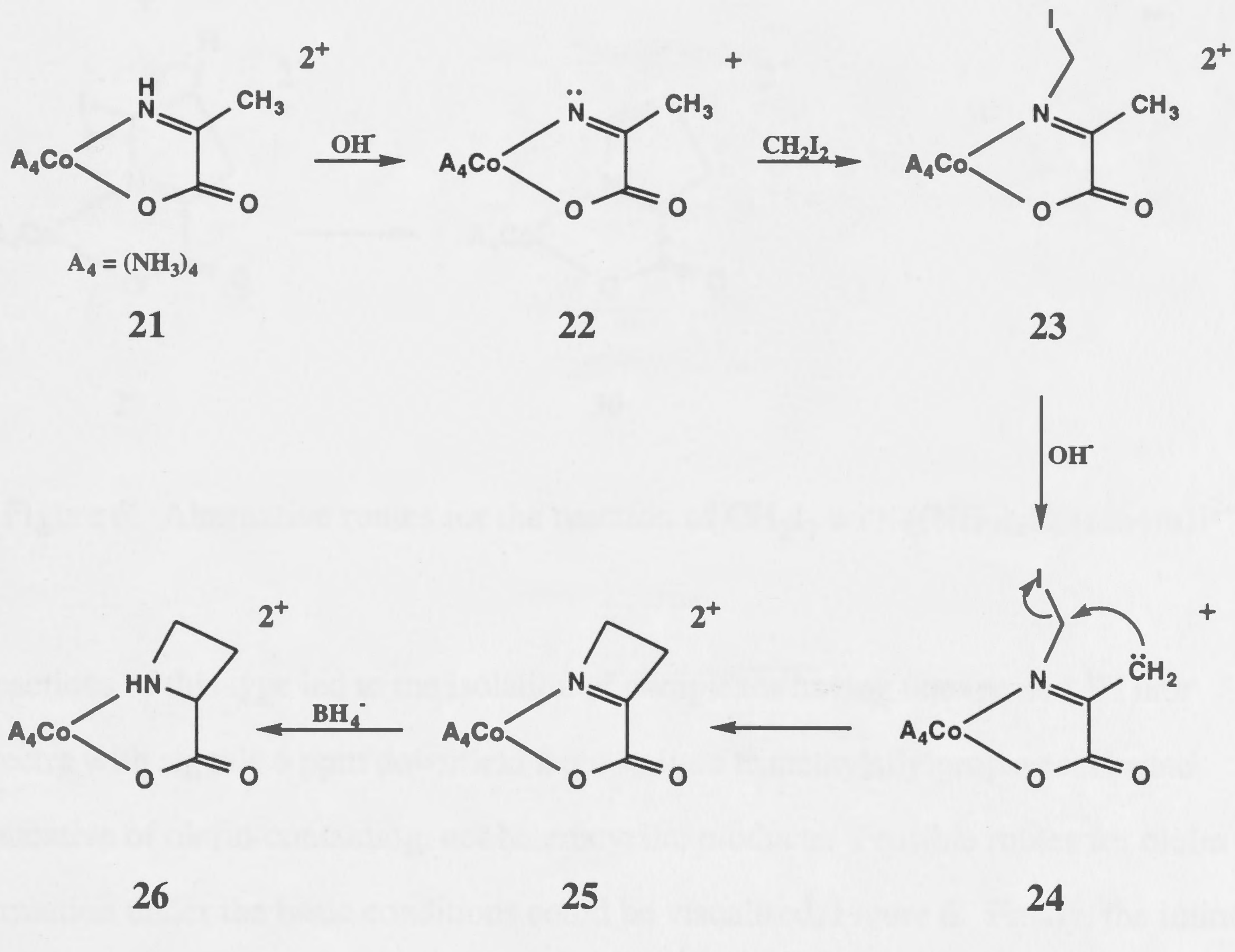



Figure 5: Sequential addition of $CH_2I_2$ to the nucleophillic imine-N and β-carbon of $[(NH_3)_4Co(ala\text{-}im)]^{2+}$.

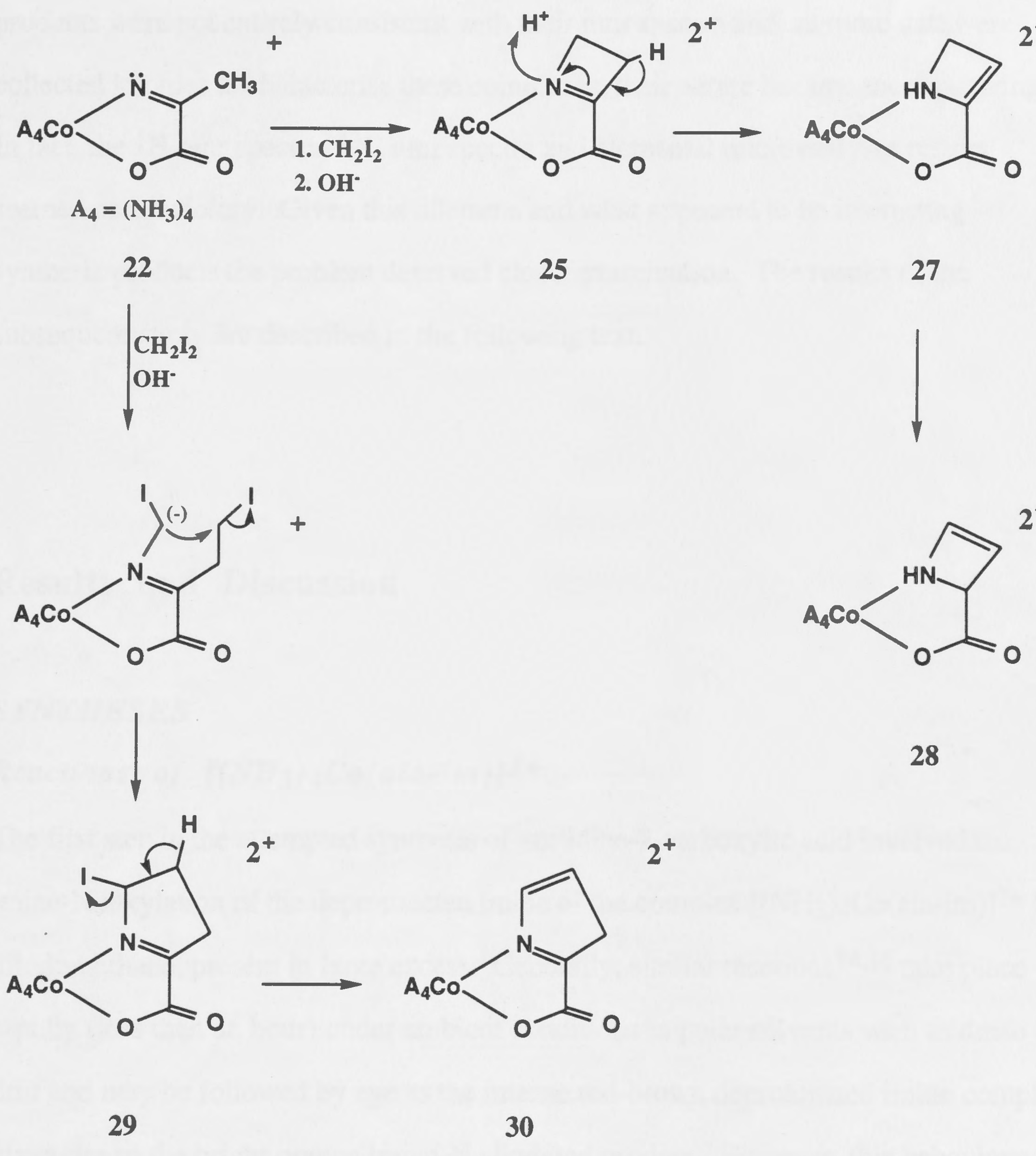


Figure 6: Alternative routes for the reaction of $CH_2I_2$ with $[(NH_3)_4Co(ala\text{-}im)]^{2+}$.

Reactions of this type led to the isolation of complexes having unexpected $^1H$ nmr spectra with signals 6 ppm downfield from sodium trimethylsilylpropanesulfonate indicative of olefin-containing, not heterocyclic, products. Feasible routes for olefin formation under the basic conditions could be visualised, Figure 6. Firstly, the imino acid could rearrange as shown, forming species such as **27** and **28**. Secondly, alkylation could occur at both the imine-N and methyl-C. The pendant haloalkane on the imine-N could then be deprotonated and the resulting carbanion displace iodide ion from the remaining pendant haloalkane, **29**. However, the structures of the proposed products were not entirely consistent with their nmr spectra and, as more data were collected in order to characterise these complexes, their nature became more puzzling. In fact, the $^{1}H$ nmr spectra, $^{13}C$ nmr spectra and elemental microanalyses results seemed contradictory. Given this dilemma and what appeared to be interesting synthetic products the problem deserved closer examination. The results of the subsequent study are described in the following text.

## Results and Discussion

### *SYNTHESES*

#### *Reactions of $[(NH_3)_4Co(ala\text{-}im)]^{2+}$*

The first step in the attempted synthesis of azetidine-2-carboxylic acid involved the imine-N alkylation of the deprotonated imine of the complex $[(NH_3)_4Co(ala\text{-}im)]^{2+}$ by diiodomethane, present in large excess. Generally, similar reactions[14,16] take place rapidly (less than an hour) under ambient conditions in polar solvents such as dmso or dmf and may be followed by eye as the intense red-brown deprotonated imine complex gives rise to the bright orange imine-N alkylated product. However, this behaviour was not observed in any of the alkylation experiments of the present study, even on extended reaction times (12 to 24 hours). Ion exchange chromatography of the resulting reaction mixtures initially produced a number of poorly resolved pink and orange bands but isolation of some of the product complexes was achieved after repeated chromatographic separations. A quantity of the (protonated) starting material, generally about 60% (after 48 hours reaction), was recovered from the reaction mixture. In addition, a complex whose colour, relatively featureless $^{1}H$ and $^{13}C$ nmr spectra and elemental microanalysis identified it as *cis* $[(NH_3)_4Co(Cl)(H_2O)]Cl_2$ was also collected, implying that there had been some loss of the imino acid during the time of the reaction. Finally, there were indications that some type of binuclear species had been synthesised. A highly charged species was eluted from the column using relatively high $H^+$ concentrations (typically 3 M HCl). However, this species decomposed in the time required to isolate it from the eluent, and so it was difficult to identify. Some other species were present in trace amounts. These were not identified, but being shades of pink, were presumed to be complexes that had partially decomposed and/or lost the chelated imino acid and/or ammonia.

One species, an orange complex present in about 10% yield, did provide an indication that a reaction of interest had occurred. Its $^1$H and $^{13}$C nmr spectra, reproduced in Figure 7, were difficult to reconcile. The $^1$H and $^{13}$C nmr of the starting material, $[(NH_3)_4Co(ala\text{-}im)]^{2+}$, are given in Figure 8 for comparison. An interpretation of the $^1$H nmr spectrum of the product, with signals at 5.79 and 6.34 ppm, was of a structure containing an olefin. These signals integrated for one proton each and $^1$H nmr decoupling experiments indicated that they were coupled to each other and to no other protons in the molecule. Similarly the signal at 3.66 ppm, which integrated for two protons, wasn't coupled to any other protons in the molecule. The peak at 2.50 ppm, due to the methyl of coordinated alanine imine (Figure 8), was replaced by a peak at 1.73 ppm consistent with attachment to saturated carbon in the product (Figure 7). Integration of this peak still indicated the presence of three protons and its position was typical of the methyl of Co(III)-coordinated alanine[19]. The inference then, was that the formation of the product included reduction of, or addition to the imine, to produce a saturated species. The three remaining, rather broad, peaks in the $^1$H nmr spectrum of Figure 7 are due to the ammonia ligands. Integration of these peaks gave a total of 9 protons not 12, but this was unexceptional because these ligands are deuterated quite rapidly in neutral $D_2O$ solutions. In summary, the $^1$H nmr spectrum of the product complex appeared to indicate that the molecule contained a methyl group, a new amine group, a methylene group and an olefin all separated from each other.

The $^{13}C$ nmr spectrum (Figure 7) contained six signals. Carboxylate carbons of Co(III) coordinated amino acids are typically found in the range 175 - 190 ppm and so the peaks at 179.9 and 186.9 ppm were ascribed to the presence of two, quite different, carboxyl groups in the product. The peak at 159.6 ppm was similarly attributed to an imine. By contrast, the imine of the starting material is positioned at 174.1 ppm (Figure 8), so a new imine must have formed in the reaction. The remaining peaks, at 62.0, 41.5, and 26.7 ppm, were identified as due to quaternary, methylene and methyl carbon atoms respectively after obtaining APT spectra. Significantly, there were no peaks in the region 110 - 140 ppm, where olefin signals would be expected to be found.[20]

It was necessary to confirm the identification of these functional groups and to correlate the proton and carbon nmr spectra of the product so a HETCOR spectrum was acquired. From this spectrum the following correlations were made:

i) protons at 1.73 ppm are attached to carbon at 26.7 ppm ($-CH_3$),

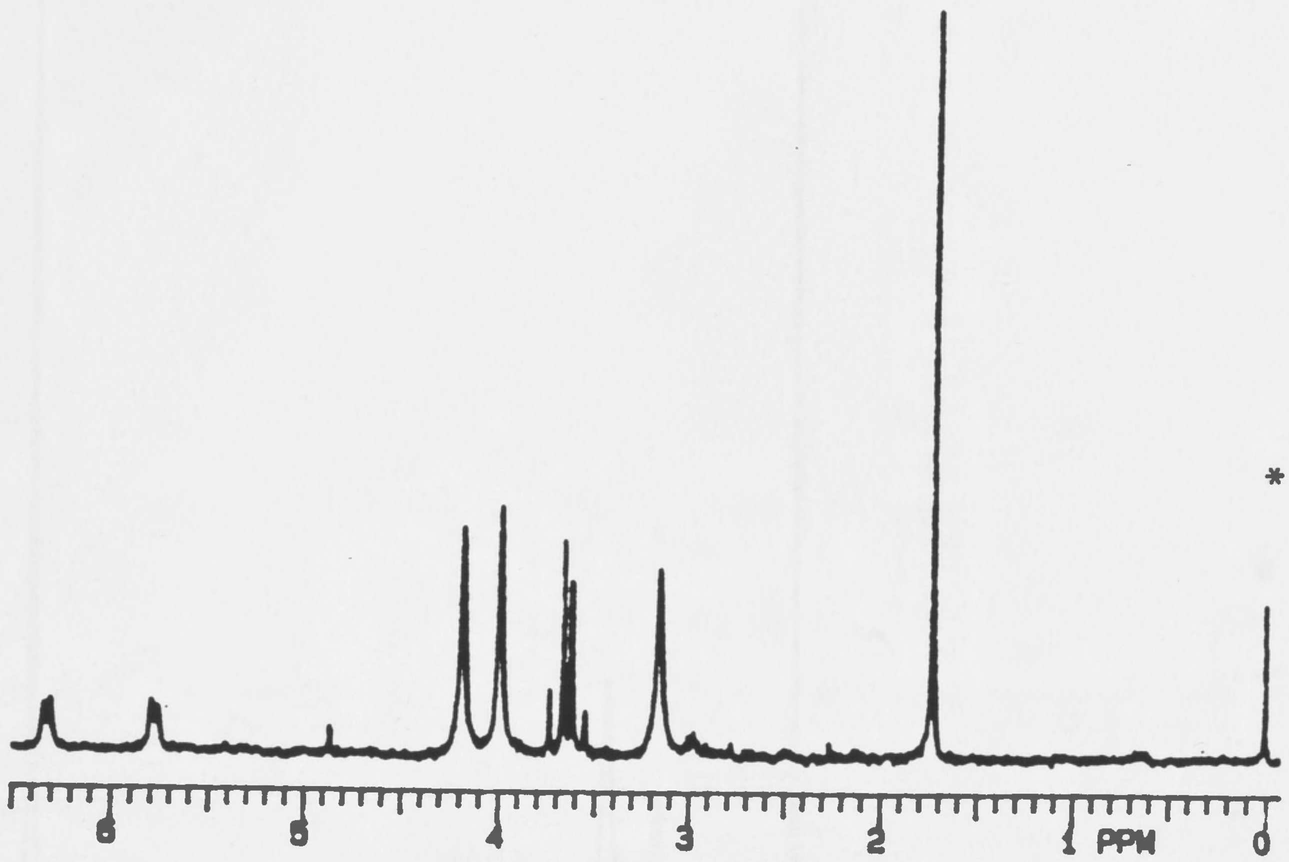
ii) protons at 3.66 ppm are attached to carbon at 41.5 ppm ($-CH_2$),

iii) carbons at 62.0, 159.6, 179.9 and 186.9 ppm are quaternary ($C_q$) and

iv) protons producing signals at 5.8 and 6.3 ppm can only be attached to nitrogen since they are not attached to any of the remaining carbon atoms ($-NH_aH_b$).

A solution of the product complex in dilute (~ 0.1M) NaOD was monitored by $^1H$ nmr spectrometry. All protons except those of the methyl and methylene groups exchanged rapidly, providing support for the hypothesis that the protons of the supposed olefin were actually attached to nitrogen.

a




b

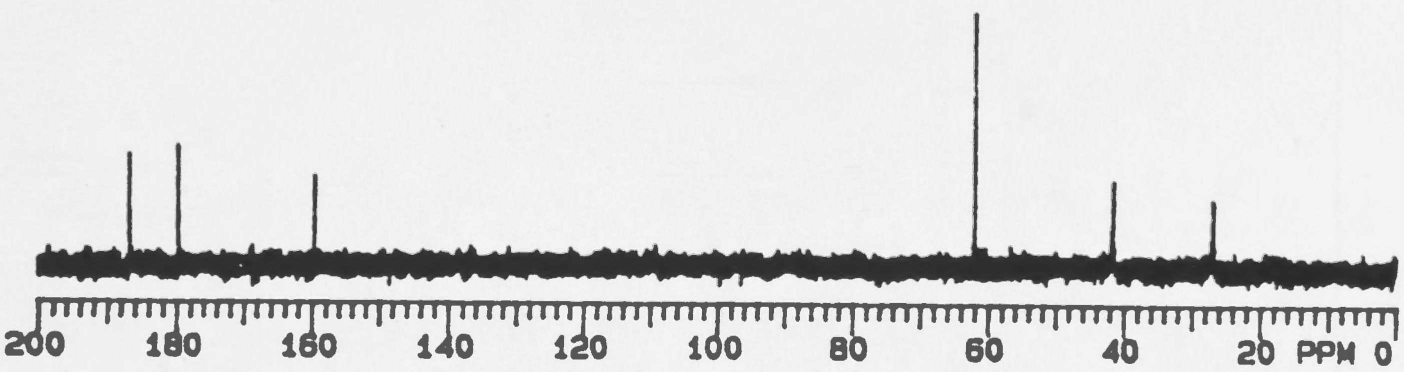



Figure 7: Nmr spectra of the low-charge species isolated from a reaction mixture containing $[(NH_3)_4Co(N{=}C(CH_3)COO)]^+$ and diiodomethane. **a**): $^1H$ nmr spectrum, 6M DCl, *NaTPS. **b**): $^{13}C$ nmr spectrum, 6M DCl, 1, 4 dioxane.

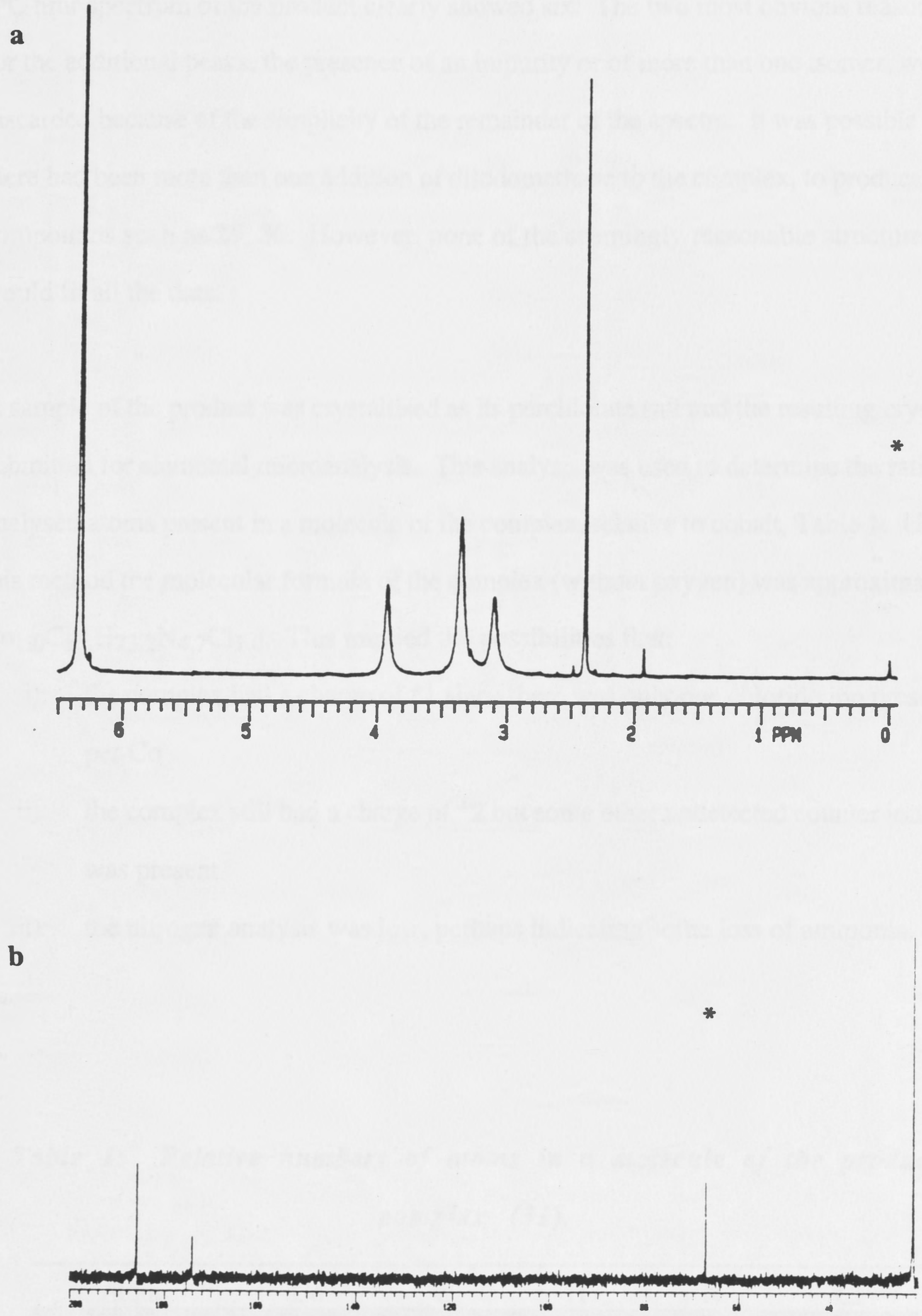



Figure 8: Nmr spectra of $[(NH_3)_4Co(NHC(CH_3)COO)]Cl_2$. **a)**: $^1H$ nmr spectrum, 6M DCl, *NaTPS. **b)**: $^{13}C$ nmr spectrum, 6M DCl, *1, 4 dioxane.

One further concern with the nmr data remained: addition of diiodomethane to the chelated imino acid should have produced a molecule having only four carbons, yet the $^{13}C$ nmr spectrum of the product clearly showed six. The two most obvious reasons for the additional peaks, the presence of an impurity or of more than one isomer, were discarded because of the simplicity of the remainder of the spectra. It was possible that there had been more than one addition of diiodomethane to the complex, to produce compounds such as **29**, **30**. However, none of the seemingly reasonable structures would fit all the data.

A sample of the product was crystallised as its perchlorate salt and the resulting crystals submitted for elemental microanalysis. This analysis was used to determine the ratio of analysed atoms present in a molecule of the complex, relative to cobalt, Table 1. Using this method the molecular formula of the complex (without oxygen) was approximately $Co_{1.0}C_{5.8}H_{23.2}N_{4.7}Cl_{1.0}$. This implied the possibilities that:

i): the complex had a charge of $^{+}1$ since there was only one chloride ion present per Co

ii): the complex still had a charge of $^{+}2$ but some other undetected counter ion was present

iii): the nitrogen analysis was low, perhaps indicating some loss of ammonia.

***Table 1: Relative numbers of atoms in a molecule of the product complex (31).***

| Atom | Co | C | H | N | Cl |
|---|---|---|---|---|---|
| Analysis (%) | 15.31 | 18.12 | 6.08 | 17.05 | 9.50 |
| No. of Atoms | 1.0 | 5.8 | 23.2 | 4.7 | 1.0 |

Crystals of **31** which were suitable for X-ray crystallographic analysis were obtained from a solution of the complex in 2 M $HClO_4$ by the addition of sodium perchlorate. The structure of the cation present in these crystals is reproduced in Figure 9. It is apparent that the deprotonated imine complex, instead of reacting with the available haloalkane, has reacted with itself by condensing a deprotonated methyl group with the $\alpha$-carbon of another molecule. Partial decomposition follows (Figure 11 illustrates the reaction pathway of the analogous ethane diamine complex). The crystal structure is of a complex with an overall charge of $^{+}2$. This is because the crystals were isolated from acidic solution and the pendant carboxyl group is protonated. If the complex is isolated from neutral solutions the carboxyl group is deprotonated and the complex has an overall charge of $^{+}1$. Bond lengths and angles of **31** are comparable to those of similar amino acid complexes[16,21,22] (average d(Co–N): 1.95 Å; d(C=N): 1.919 Å). Selected bond lengths are given in Tables 2 and 3; the remainder are tabulated in the appendices.

The nmr data were assigned to structure **31** (Table 4). The addition of a second molecule of alanine imine to the first provided a sufficiently constrained environment that the geminal protons of the new primary amine at N(1) are quite different from each other and have quite different chemical shifts. These signals are quite sharply defined and are not broadened much by the N-quadrupolar coupling. The proton on the new imine at N(2) did not appear in spectra measured in $D_2O$ and DCl but was present at 11.45 ppm (relative to dioxane 3.74 ppm) in spectra of samples dissolved in $d_6$-dmso.

The self condensation of the complex was demonstrated to occur in dmso in the absence of $CH_2I_2$. The reaction was also attempted under aqueous conditions, without success. The only products from the reaction were the protonated starting material and Co(II). In part, this is because tetraammine complexes are less stable in basic aqueous solution. It is also probable that the initial addition of one complex to another is accomplished, but the binuclear species so formed is hydrolysed before the intramolecular condensation, with one of the ammonia ligands, occurs.

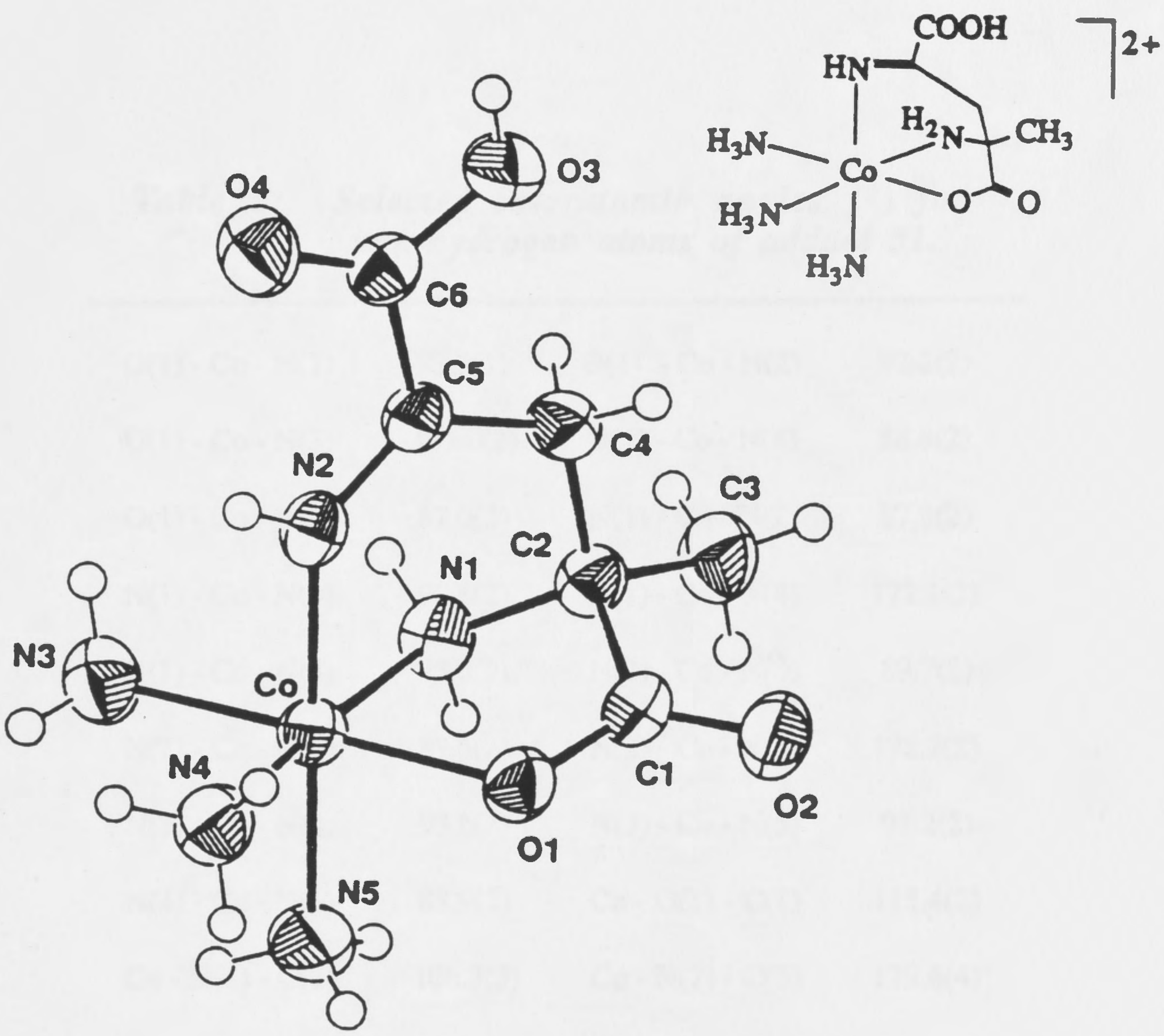



Figure 10: ORTEP diagram of the cation in the crystal structure of $[(NH_3)_3Co(C_6H_9N_2O_4)](ClO_4)_2$, **31**. Ellipsoids show 50% probability levels and hydrogen atoms have been drawn as circles of arbitrary small radius.

***Table 2: Selected interatomic distances (Å) for non-hydrogen atoms of adduct 31.***

| | | | |
|---|---|---|---|
| Co–O(1) | 1.891(3) | O(1)–C(1) | 1.280(6) |
| Co–N(1) | 1.944(4) | O(2)–C(1) | 1.222(5) |
| Co–N(2) | 1.919(4) | O(3)–C(6) | 1.303(7) |
| Co–N(3) | 1.964(4) | O(4)–C(6) | 1.200(7) |
| Co–N(4) | 1.959(4) | N(1)–C(2) | 1.500(6) |
| Co–N(5) | 1.973(6) | N(2)–C(5) | 1.273(6) |

***Table 3: Selected interatomic angles (°) for non-hydrogen atoms of adduct 31.***

| | | | |
|---|---|---|---|
| O(1) - Co - N(1) | 83.9(1) | O(1) - Co - N(2) | 92.2(2) |
| O(1) - Co - N(3) | 177.0(2) | O(1) - Co - N(4) | 88.8(2) |
| O(1) - Co - N(5) | 87.0(2) | N(1) - Co - N(2) | 87.8(2) |
| N(1) - Co - N(3) | 93.8(2) | N(1) - Co - N(4) | 172.1(2) |
| N(1) - Co - N(5) | 93.6(2) | N(2) - Co - N(3) | 89.7(2) |
| N(2) - Co - N(4) | 89.5(2) | N(2) - Co - N(5) | 178.2(2) |
| N(3) - Co - N(4) | 93.6(2) | N(3) - Co - N(5) | 91.2(2) |
| N(4) - Co - N(5) | 88.9(2) | Co - O(1) - C(1) | 115.4(2) |
| Co - N(1) - C(2) | 106.3(3) | Co - N(2) - C(5) | 129.6(4) |
| O(1) - C(1) - O(2) | 122.7(4) | O(1) - C(1) - C(2) | 115.4(4) |
| O(2) - C(1) - C(2) | 121.8(5) | N(1) - C(2) - C(1) | 104.8(4) |
| N(1) - C(2) - C(3) | 110.8(4) | N(1) - C(2) - C(4) | 109.9(4) |
| C(1) - C(2) - C(3) | 112.4(4) | C(1) - C(2) - C(4) | 108.7(4) |
| C(3) - C(2) - C(4) | 110.2(4) | C(2) - C(4) - C(5) | 116.7(4) |
| N(2) - C(5) - C(4) | 123.2(5) | N(2) - C(5) - C(6) | 117.0(4) |
| C(4) - C(5) - C(6) | 119.8(4) | O(3) - C(6) - O(4) | 125.6(5) |
| O(3) - C(6) - C(5) | 113.2(4) | O(4) - C(6) - C(5) | 121.2(5) |

***Table 4: Assignment of nmr data to Adduct 31***

| Atom | $^{1}H$ nmr[1] | $^{13}C$ nmr[2] |
|---|---|---|
| $-N(1)H_2$ | 5.79, 6.34 | – |
| $=N(2)H$ | * | – |
| $-N(3,4,5)H_2$ | 4.18, 3.99, 3.15 | – |
| $-C(1)O_2$ | – | 179.86 |
| -qC(2) | – | 61.99 |
| $-C(3)H_3$ | 1.73 | 26.89 |
| $-C(4)H_2$ | 3.66 | 41.50 |
| -C(5)=N | – | 159.63 |
| $-C(6)O_2$ | – | 186.85 |

[1] 6M DCl, NaTPS (0.00 ppm) as internal standard. [2] 6M DCl, dioxane (3.74 ppm) as internal standard. * deuteriated under conditions of experiment.

### *Self Condensation of $[(en)_2Co(ala\text{-}im]^{2+}$*

Similar synthetic experiments (with and without diiodomethane) were performed using the corresponding bis (ethane diamine) complex, $[(en)_2Co(N{=}C(CH_3)COO)]ClO_4$. Again self condensation of the coordinated imino acid occurred in preference to imine-N alkylation by an available haloalkane. An orange complex, **32a**, was isolated, in comparable yield and having $^{1}H$ and $^{13}C$ nmr spectra similar to those of the tetraammine complex, Figure 10.

The mechanism by which $[N_4Co(ala\text{-}im)]^{2+}$ condenses with itself is depicted in Figure 11, using $[(en)_2Co(ala\text{-}im)]^{2+}$ as an example of the substrate. The mechanism of self condensation of $[(NH_3)_4Co(ala\text{-}im)]^{2+}$ can be expected to be the same, with the exception that it is deprotonated ammonia, not deprotonated ethane diamine, that takes part in the reaction.

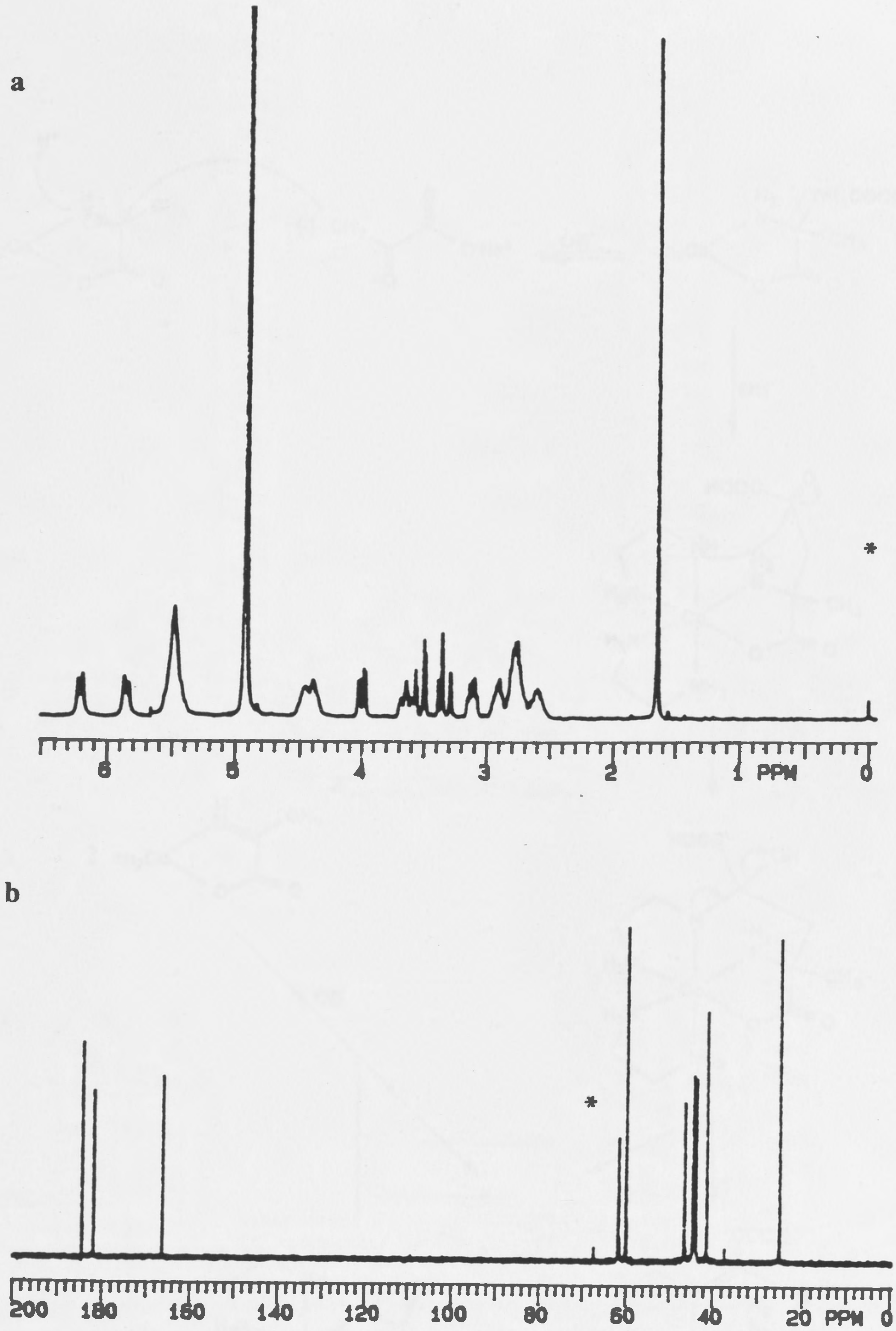



Figure 10: Nmr spectra of **32a**. **a):** $^{1}H$ nmr spectrum, 6M DCl, *NaTPS.
**b):** $^{13}C$ nmr spectrum, 6M DCl, *1, 4 dioxane. The product was separated from the reaction mixture by chromatography and the residue from the band containing all possible **32** was used in making the nmr sample.

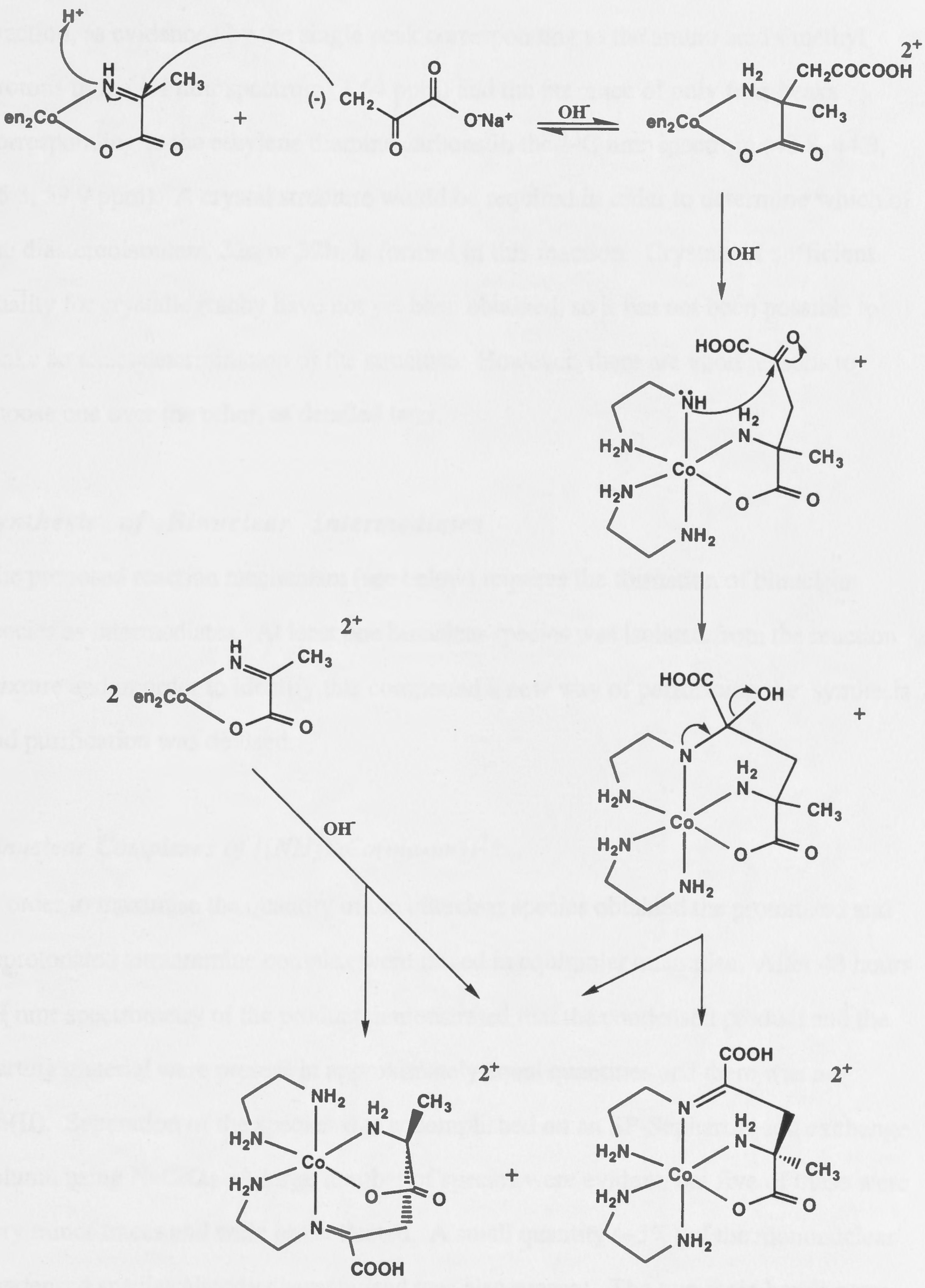



Figure 11: Mechanism of the self condensation of $[(en)_2Co(ala\text{-}im)]^{2+}$ in dmso.

There are two possible diastereoisomers (and their attendant enantiomers) of **32a** and **b**, Figure 11. However, only one pair of these was found in the product of the reaction, as evidenced by the single peak corresponding to the amino acid's methyl protons in the $^{1}H$ nmr spectrum (1.64 ppm) and the presence of only four peaks corresponding to the ethylene diamine carbons in the $^{13}C$ nmr spectrum (43.8, 44.3, 46.3, 59.9 ppm). A crystal structure would be required in order to determine which of the diastereoisomers, **32a** or **32b**, is formed in this reaction. Crystals of sufficient quality for crystallography have not yet been obtained, so it has not been possible to make an exact determination of the structure. However, there are good reasons to choose one over the other, as detailed later.

### *Synthesis of Binuclear Intermediates*

The proposed reaction mechanism (see below) requires the formation of binuclear species as intermediates. At least one binuclear species was isolated from the reaction mixture and in order to identify this compound a new way of performing the synthesis and purification was devised.

#### *Binuclear Complexes of $[(NH_3)_4Co(ala\text{-}im)]^{2+}$*

In order to maximise the quantity of the binuclear species obtained the protonated and deprotonated tetraammine complex were mixed in equimolar quantities. After 48 hours $^{1}H$ nmr spectrometry of the product demonstrated that the condensed product and the starting material were present in approximately equal quantities and there was no Co(II). Separation of the species was accomplished on an SP-Sepharose ion exchange column using $NaClO_4$. A large number of species were evident, but five of these were very minor traces and were not collected. A small quantity (~5%) of the mononuclear condensed species already characterised was also present. The two main bands were the starting material which comprised 40% of the total material and a polynuclear metal complex which accounted for ~40% of the material. Much of the $NaClO_4$ in the eluent of this band was removed by crystallisation as the solution was evaporated; more was removed by passing the resulting solution down a column of size exclusion resin (Biogel P2). The eluent from this column was used to obtain a $^{13}C$ nmr spectrum, Figure 12. There are the same number of carbon atoms present in this spectrum as in that of the final product, Figure 7. The presence of two carbonyl signals and one imine signal in the spectrum of the binuclear complex implies a structure such as **33**. The peak positions for all but the imine carbon, C(5), do not move upfield by more than 2 ppm in the formation of the final product. Attempts to purify the binuclear metal species for $^1H$ and $^{59}Co$ nmr spectrometry were unsuccessful. The complex degraded continuously to the (condensed) mononuclear species in the process.

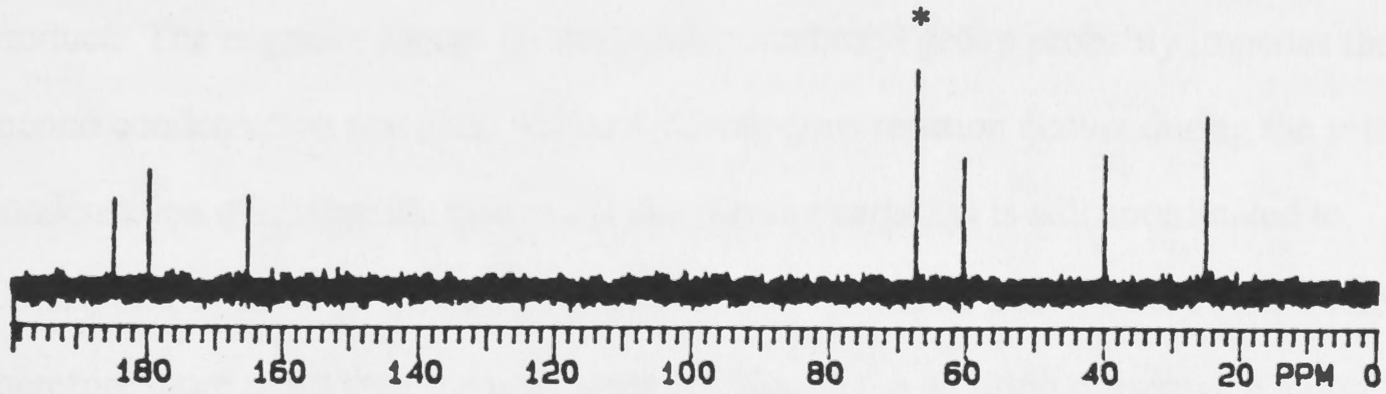



Figure 12: $^{13}C$ nmr spectrum of the binuclear species **33**. $H_2O/D_2O$, *dioxane.

### *Binuclear Complexes of $[(en)_2Co(ala\text{-}im)]^{2+}$*

In a manner similar to that described above, binuclear intermediates of the self condensation of $[(en)_2Co(ala\text{-}im)]^{2+}$ were synthesised from equimolar quantities of the protonated and deprotonated starting materials. The binuclear species which resulted were stable over a wider pH range and for longer periods of time and were partially purified on Dowex ion exchange resin, with an acid eluent. The $^{1}H$ and $^{13}C$ nmr spectra of the binuclear materials are reproduced in Figure 13. It appeared that the product contained a number of the possible diastereoisomers. Attempts to separate these isomers by chromatography were unsuccessful because the binuclear species hydrolysed to the mononuclear product when left in aqueous solutions over time.

### ***Condensation of sodium pyruvate with $[(en)_2Co(ala\text{-}im)]^{2+}$***

Another way of synthesising the mononuclear condensation products **31** and **32a** would be to add pyruvate to the imino acid complexes in a two step procedure, Figure 11. When sodium pyruvate was added to $[(en)_2Co(ala\text{-}im)]^{2+}$ in $NaHCO_3/Na_2CO_3$ buffer the condensation proceeded smoothly. The second step of the reaction was accomplished by leaving the pyruvate adduct in carbonate buffer for an extended period of time. On work up two complexes were isolated: a small quantity of the condensed monomer **32a** and the imino acid, $[(en)_2Co(ala\text{-}im)]^{2+}$. Under the conditions of the experiment the addition of pyruvate appears to be reversible and hydrolysis of the pyruvate fragment is faster than the Schiff base reaction which gives the desired product. The negative charge on the pendant carboxyl group probably impedes the second condensation reaction. When the analogous reaction occurs during the self condensation of chelate alanine imine the pendant carboxyl is still coordinated to Co(III) and so its negative charge is neutralised. The final Schiff base condensation is therefore more rapid than the analogous reaction in the addition of pyruvate anion.

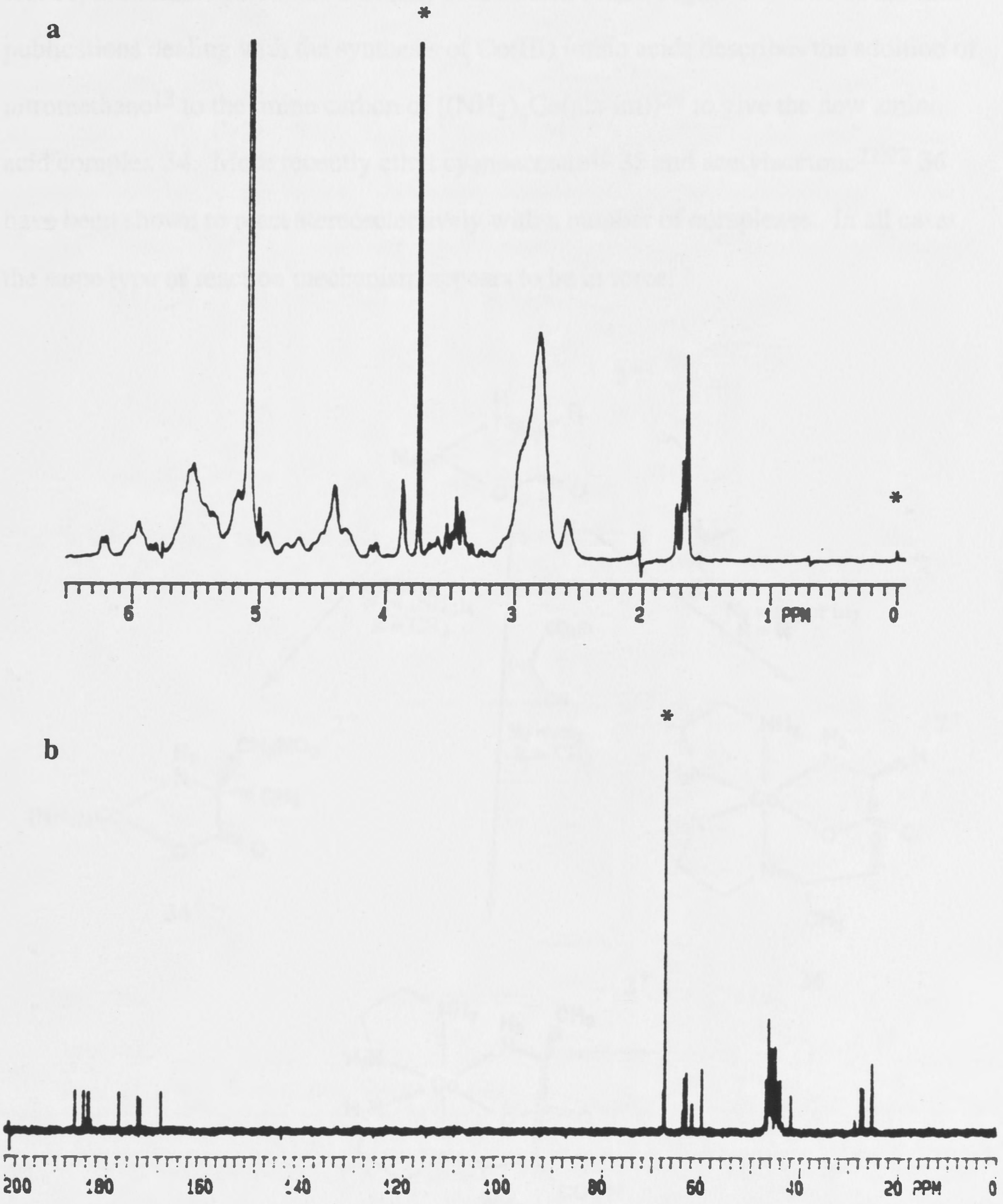



Figure 13: Nmr spectra of binuclear intermediates in the self condensation of $[(en)_2Co(ala\text{-}im)]^{2+}$. **a**): $^1H$ nmr spectrum, 0.1M DCl, *NaTPS. **b**): $^{13}C$ nmr spectrum, 0.1M DCl, *1, 4 dioxane.

## *REACTION MECHANISM*

There are a number of examples in the literature of carbanion addition to the electrophilic imine carbon of a Co(III) coordinated imine, Figure 14. One of the first publications dealing with the synthesis of Co(III) imino acids describes the addition of nitromethane[13] to the imine carbon of $[(NH_3)_4Co(\text{ala-im})]^{2+}$ to give the new amino acid complex **34**. More recently ethyl cyanoacetate[16] **35** and acetylacetone[21,22] **36** have been shown to react stereoselectively with a number of complexes. In all cases, the same type of reaction mechanism appears to be in force.

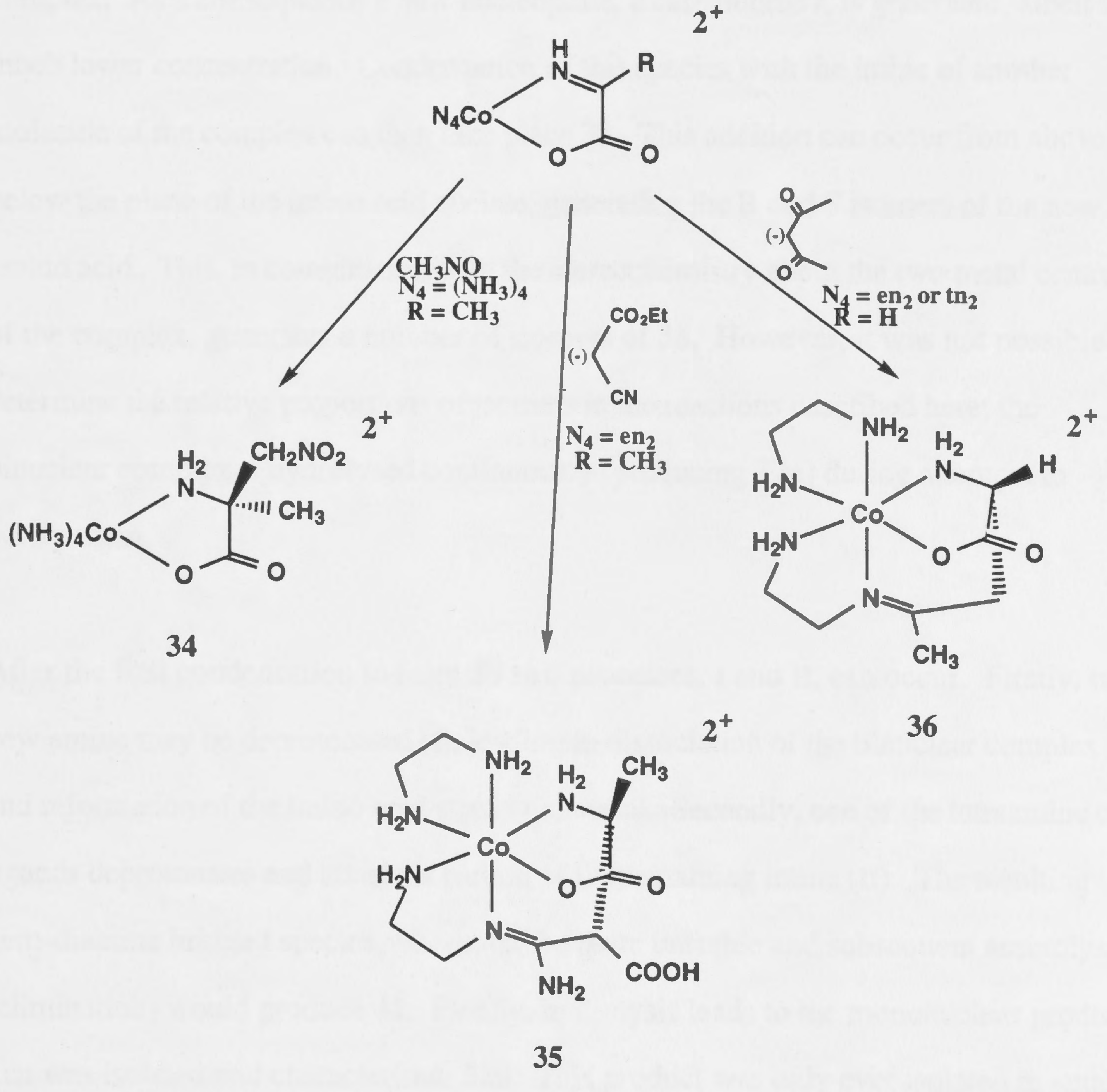



Figure 14: Examples of nucleophillic addition to the α-carbon of Co(III) complexes of imino acids.

First, there is nucleophilic attack of the carbanion, formed in the basic conditions of the experiment, on the susceptible carbon of the imine. This is followed by a second condensation, this time with a nucleophile generated by the deprotonation of an amine group of the complex's coligands, at a second reactive site on the original carbanion.

Figure 15 represents the reaction mechanism involved in the self condensation of $[(en)_2Co(ala\text{-}im)]^{2+}$. Under the basic conditions of these experiments, the deprotonated imine ($pK_a \sim 10$) may scavenge protons from different sources, including from the methyl group of its own imino acid ($pK_a \sim 14$) or from that of another complex. As a consequence a new nucleophile, a carbanion **37**, is generated, albeit in much lower concentration. Condensation of this species with the imine of another molecule of the complex can then take place **38**. This addition can occur from above or below the plane of the imino acid chelate, generating the R and S isomers of the new amino acid. This, in combination with the stereochemistry about the two metal centres of the complex, generates a number of isomers of **38**. However, it was not possible to determine the relative proportions of isomers in the reactions described here; the binuclear complexes hydrolysed continuously (producing **32a**) during attempts to purify them.

After the first condensation to form **38** two processes, I and II, can occur. Firstly, the new amine may be deprotonated (I), leading to dissociation of the binuclear complex and reformation of the imino acid starting material. Secondly, one of the tetraamine co-ligands deprotonates and attacks a carbon of the remaining imine (II). The resulting gem-diamine bridged species, **40**, should be quite unstable and subsequent aminolysis (elimination) would produce **41**. Finally, hydrolysis leads to the mononuclear product that was isolated and characterised, **32a**. This product was only ever isolated in small quantities (5 - 10 % of the total material), implying that the rate of its formation was slow compared to the rate of formation and hydrolysis of the first binuclear intermediate to the reactant.

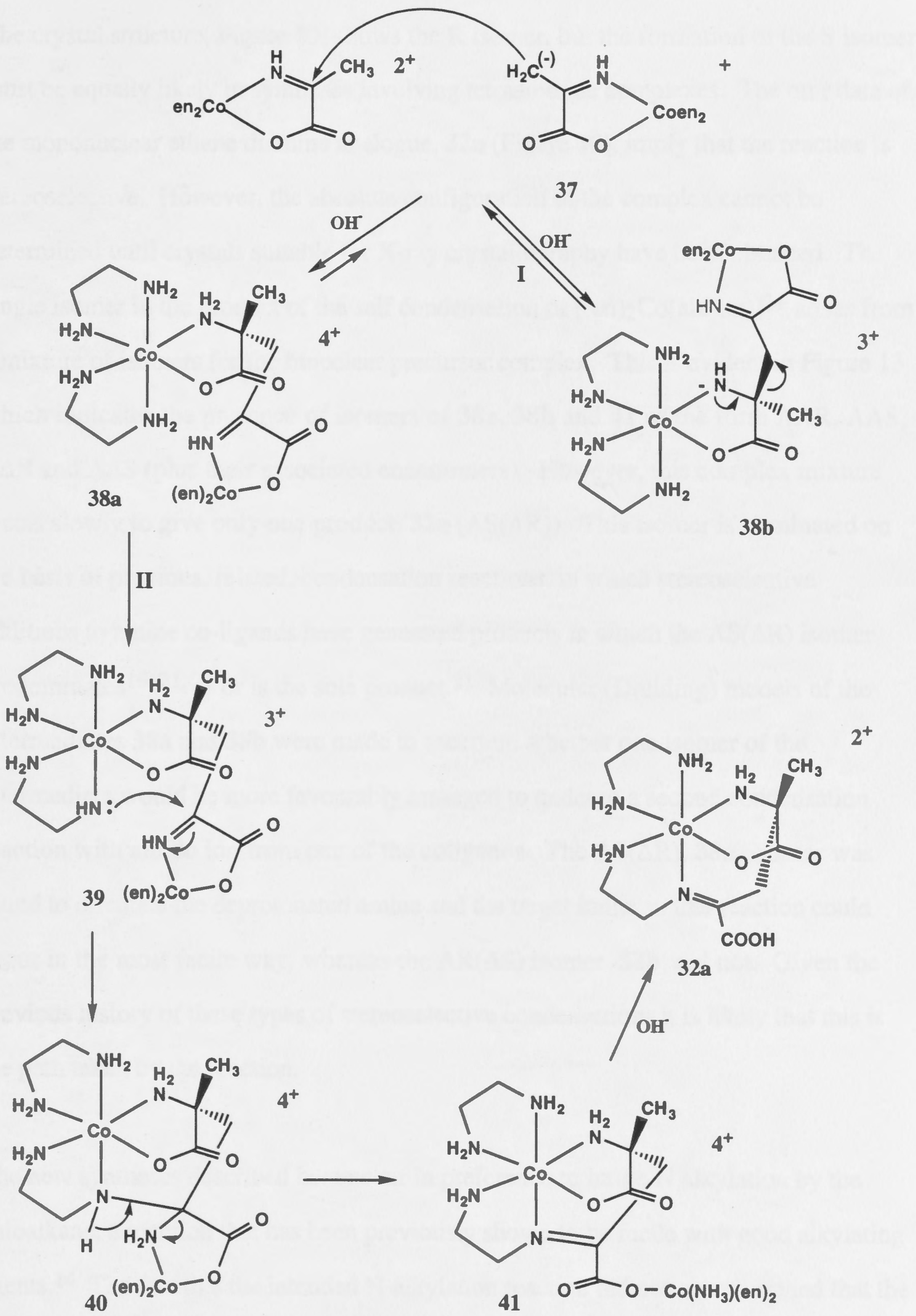


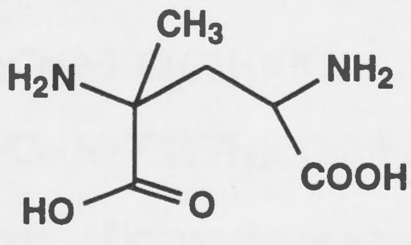

Figure 15: Stereoselective self condensation of $[(en)_2Co(ala\text{-}im)]^{2+}$.

The crystal structure, Figure 10, shows the R isomer, but the formation of the S isomer must be equally likely in syntheses involving tetraammine complexes. The nmr data of the mononuclear ethane diamine analogue, **32a** (Figure 10), imply that the reaction is stereoselective. However, the ~~absolute~~ configuration of the complex cannot be determined until crystals suitable for X-ray crystallography have been obtained. The single isomer in the product of the self condensation of $[(en)_2Co(ala\text{-}im)]^{2+}$ arises from a mixture of isomers for the binuclear precursor complex. This is evident in Figure 13 which indicates the presence of isomers of **38a**, **38b** and **41** of the form ΛΛR, ΛΛS, ΛΔR and ΛΔS (plus their associated enantiomers). However, this complex mixture reacts slowly to give only one product, **32a** (ΛS(ΔR)). This isomer is nominated on the basis of previous, related, condensation reactions, in which stereoselective additions to amine co-ligands have generated products in which the ΛS(ΔR) isomer predominates[16, 21, 22] or is the sole product.[21] Molecular (Dreiding) models of the intermediates **38a** and **38b** were made to ascertain whether one isomer of the intermediate would be more favourably arranged to undergo a second condensation reaction with amido ion from one of the coligands. The ΛS(ΔR), **38a,** isomer was found to orientate the deprotonated amine and the target imine so that reaction could occur in the most facile way, whereas the ΛR(ΔS) isomer, **38b**, did not. Given the previous history of these types of stereoselective condensations it is likely that this is the path taken by the reaction.

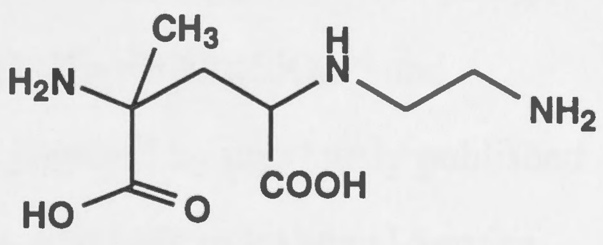
The new syntheses described here occur in preference to imine-N alkylation by the haloalkane, a reaction that has been previously shown to be facile with good alkylating agents.[14] The fact that the intended N-alkylation reaction did not occur implied that the rate of the addition of the alkyl halide, to generate **23**, was very slow, relative to the rate of addition of the alanine imine carbanion to the imine carbon. Previous N-alkylation reactions[14,16] involved more reactive reagents such as iodomethane, allylbromide and benzylbromide. The diiodo- derivatives are more sterically hindered and undergo nucleophilic substitution more slowly as a result. If the N-alkylation reaction is slow then self-condensation by the deprotonated imino acid becomes competitive.

The deprotonated imine is potentially a very useful reagent for the synthesis of novel heterocyclic amino acids and some experiments, using different haloalkanes, have been performed in order to extend the range of possible imino acids available from such alkylation reactions. These are described in the following chapter.

The self condensation reaction described in this chapter, along with other reactions which feature reactions with both the α-carbon and coligand-N of imino acid complexes, provide a means of synthesising new amino acids with polyfunctional side chains. These may be modified further or the imine moiety may be reduced and the amino acids (**42**, **43**) isolated from the complex as has been described in previous chapters.




**42**




**43**

## Experimental

### *INSTRUMENTS, REAGENTS AND ANALYSES*

$^1$H and $^{13}$C nuclear magnetic resonance spectra of the complexes dissolved in $D_2O$, 0.1 M DCl, 2 M DCl, 6 M DCl, 0.1 M NaOD or $d_6$-dmso were acquired using a Varian

Instruments Gemini 300 NMR spectrometer. HETCOR spectra were acquired using a Varian Instruments VXR 300 NMR spectrometer. Chemical shifts in $^{1}H$ nmr spectra are reported relative to sodium trimethylsilylpropanesulfonate (NaTPS), 0.00 ppm. Chemical shifts in $^{13}C$ nmr were established relative to dioxane, 67.4 ppm.

Multiplicities of signals in the $^{1}H$ nmr spectra are indicated by the following abbreviations: Singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), AB spin system (AB), AX spin system (AX). Most solvents and basic chemicals used for syntheses were analytical reagent grade. Commercial $CF_3SO_3H$ was distilled before use. Dmf and dmso were dried over $CaSO_4$ before use. Ion exchange chromatography was performed with analytical grade Dowex 50Wx2 ($H^+$ form, 200 - 400 mesh, Bio-Rad), SP Sephadex C25 ($Na^+$ form, Pharmacia), or S Sepharose ($Na^+$ form, 45 - 165 μm wet, Pharmacia) cation exchange resins. Size exclusion chromatography was performed using Biogel P2 (200 - 400 mesh). Complexes present in the collected eluents were recovered by evaporation under water pump vacuum (~ 20 τ) on a Büchi rotary evaporator, with a water bath temperature of less than 40 °C. The complexes $[(NH_3)_4Co(NHC(CH_3)COO)]Cl_2$[14], $[(NH_3)_4Co(NC(CH_3)COO)]ClO_4$[14], $[(NH_3)_4Co(NHC(CH_3)COO)](CF_3SO_3)_2$[23], $[(en)_2Co(NHC(CH_3)COO)]Cl_2$,[15] $[(en)_2Co(NC(CH_3)COO)]ClO_4$[14] and $[(en)_2Co(NHC(CH_3)COO)](CF_3SO_3)_2$[23] were prepared by previously published methods. Microanalyses were performed by the ANU Microanalytical Service.

## *SYNTHESES*

### *Attempted syntheses of $[(NH_3)_4Co(NRC(CH_3)COO)]^{2+}$, R = $CH_2I$*

In a typical experiment, $[(NH_3)_4Co(NC(CH_3)COO)]ClO_4$, (0.75 g) was dissolved in dmso, (10 $cm^3$). Diiodomethane (6.65 g) was added to the resulting deep brown-orange solution and the mixture stirred at room temperature for 2 hours. After this time there was no change to the colour of the solution and the reaction was quenched by the addition of $H_2O$ (100 $cm^3$), to form an orange solution. Excess diiodomethane was removed by washing the aqueous phase with dichloromethane (2 x 50 $cm^3$). The aqueous phase was then applied to an SP Sephadex column (2.5 x 17.0 cm). The adsorbed material was washed with $H_2O$ before eluting with 0.075 M $NaH_2PO_4$ (pH 5.5). Three orange bands separated on the column during elution:

*Band 1:* a low-charge species, subsequently identified as the condensed monomer **31.** This material usually comprised about 10% of the total material retrieved from the column.

*Band 2:* the (protonated) starting material. In variations of the above experiment this complex accounted for about 60% of the material on the column.

*Band 3:* a highly charged species, identified as a dimer with the proposed structure **33**. This generally amounted to about 20% of the material on the column.

Traces of pink-coloured complexes were also removed from the column. Their colour implied that they were no longer complexes with $N_5O$ type donor sets. Nuclear magnetic resonance spectra and elemental microanalysis of those bands which provided sufficient material identified them to be species such as $[(NH_3)_4CoCl(OH_2)]Cl_2$. The complex from *Band 1* was desalted on a small bed of Dowex resin; after washing the adsorbed material with $H_2O$ and 0.5 M HCl it was eluted with 1 M HCl. The acid was removed by rotary evaporation and the residue that remained was recrystallised twice from water with the addition of a little ethanol and refrigeration. The product was an orange microcrystalline solid (0.05 g, 11%) The pendant carboxyl group in the isolated complex was deprotonated, to give an overall charge of $^{+}1$. Analysis calculated for $[CoC_6H_{17}N_5O_8Cl].0.5H_2O$: Co, 15.09; C, 18.45; H, 4.65; N, 17.93; Cl, 9.08. Found: Co, 15.3; C, 18.1; H, 6.1; N, 17.1; Cl, 9.5. $^1H$ nmr (6 M DCl): $\delta$ 6.33, 5.79 (AXq, 2H, $NH_2$), 4.18 (br, 3H, $NH_3$), 3.99 (br, 3H, $NH_3$), 3.66 (AB q, 2H, $CH_2$), 3.15 (br, 3H, $NH_3$), 1.73 (s, 3H, $CH_3$). $^{13}C$ nmr (6 M DCl): $\delta$ 186.6 (COO), 179.9 (Co-COO), 159.6 (C=N), 62.0 ($C_q$), 41.5 ($CH_2$), 26.9 ($CH_3$).

Crystals suitable for X-ray crystallography were grown by dissolving some of the chloride salt in 6 M $HClO_4$ and chilling the solution in an ice bath. This resulted in a complex in which the pendant carboxyl group was protonated. Analysis calculated for $[CoC_6H_{20}N_5O_{14}Cl_2].2H_2O$: Co, 11.42; C, 13.96; H, 3.91; N, 13.57; Cl, 13.74. Found: Co, 11.2; C, 14.0; H, 4.1; N, 13.3; Cl, 13.9. $^1H$ and $^{13}C$ nmr spectra are described above.

The material from *Band 3* was desalted on Dowex as described above. However, on rotary evaporation a pink complex precipitated from the orange solution and it was not possible to recrystallise the remaining material satisfactorily. The pink material was identified by its almost featureless nmr spectra and its elemental microanalysis as $[(NH_3)_4Co(OH_2)Cl]Cl_2$.

Different solvents (dmso, dmf and acetonitrile) and dihaloalkanes ($X(CH_2)_nX$; X = Br, I; n = 1, 2, 3) were used in successive experiments without changing the nature or the proportions of the products to any great degree. The reaction time was increased to a total of 24 hours over which time there was a slight increase in the proportion of the monomer isolated from the reaction mixture. Subsequent to the identification of the monomer, the reaction was performed in the absence of any alkylating reagent to produce the same mixture of complexes.

***Self condensation of $[(NH_3)_4Co(ala\text{-}im)]Cl_2$ in aqueous conditions***

The complex $[(NH_3)_4Co(NHC(CH_3)COO)]Cl_2$ (0.03 g) was dissolved in carbonate buffer ($[CO_3^{2-}] = [HCO_3^-] = 0.5$ M, 1 $cm^3$). Samples of the resulting red solution were taken over the next 90 minutes and adsorbed on Sephadex columns (0.5 x 4 cm). These were eluted with 0.5 M NaCl and the behaviour of the complexes adsorbed on them compared with the behaviour of authentic samples of the starting material and the condensed monomer **31**. Only the starting material was observed on the columns.

After 14 hours only the starting material and Co(II) (identified with Co(II) indicator strips) were present. No indications of condensed species were seen.

***Isolation and characterisation of an ethanediamine analogue, [(en)Co(NH$_2$CH$_2$CH$_2$NC(COOH)CH$_2$C(NH$_2$)(CH$_3$)COO))]Cl$_2$ (32)***

In a similar manner to the experiments described above, [(en)$_2$Co(NC(CH$_3$)COO)]ClO$_4$ (0.50 g) was dissolved in dmso (10 cm$^3$) and the resulting deep brown solution left to stir at ~ 20 °C for 2.5 hours. After this time the reaction was quenched by diluting the reaction mixture with $H_2O$ (100 cm$^3$) and the orange solution passed through a column of Sephadex (2.5 x 19 cm). The adsorbed material was washed with $H_2O$ and eluted using a phosphate buffer ([Na$_2$HPO$_4$] = 0.05 M, [NaH$_2$PO$_4$] = 0.1 M, pH = 6.5) to separate the mixture on the column. Four orange bands formed:

*Band 1:* pale orange, about 5 - 10% of the total material. It was adsorbed on a small bed of Dowex, washed with water and 0.5 M HCl and eluted with 2 M HCl. The acid was removed by rotary evaporation and the solid remaining recrystallised from dilute HCl by the addition of ethanol. The product was an orange microcrystalline solid (0.05 g, 9%) and was identified as the condensed, monomeric species, **32**. Analysis calculated for [CoC$_{10}$H$_{20}$N$_5$O$_4$Cl$_2$].2H$_2$O: Co, 14.48; C, 29.53; H, 6.44; N, 17.22; Cl, 8.72. Found: Co, 13.7; C, 29.5; H, 6.3; N, 17.3; Cl, 7.5. $^1$H nmr (0.1 M DCl): δ 6.20, 5.85 (AXq, 2H, NH$_2$), 5.48 (br, 4H, 2 x NH$_2$), 4.40 (br, 2H, NH$_2$), 4.02 (dd, 1H, CH-N=C), 3.98 (m, 1H, CH-N=C), 3.43 (ABq, 2H, CH$_2$-C=N), 3.11, 2.74-2.91 (m, 6H, en-CH$_2$), 1.64 (s, 3H, CH$_3$). $^{13}$C nmr (0.1 M DCl): δ 184.4 (COO), 181.8 (Co-COO), 166.3 (C=N), 61.6 (C$_q$), 59.9 (CH$_2$-N=), 46.3 (en-CH$_2$), 44.3 (en-CH$_2$), 43.8 (en -CH$_2$), 41.3 (CH$_2$), 25.0 (CH$_3$).

*Band 2:* was identified by its nmr spectra as the (protonated) starting material.

*Band 3* + *Band 4:* did not completely separate. The material obtained after desalting was a mixture of products that was never successfully separated. It has been presumed that the binuclear species **33** was present since some of the corresponding monomer was subsequently isolated from the mixture.

***Synthesis of the binuclear complex formed by the self condensation of*** $[(NH_3)_4Co(ala\text{-}im)]^{2+}$

$[(NH_3)_4Co(NC(CH_3)COO)]ClO_4$ (0.30 g) and the corresponding (protonated) $[(NH_3)_4Co(NHC(CH_3)COO)](CF_3SO_3)_2$ (0.50 g) were dissolved in dmso (10 $cm^3$). The resulting deep brown-orange solution was stirred at room temperature for 48 hours. A few drops of glacial acetic acid were added to quench the reaction and then the solution was dripped into stirred diethyl ether (300 $cm^3$), forming a red oil. The ether was decanted and the oil dissolved in a minimum volume of acetone. A pink precipitate that did not dissolve was filtered and discarded before the acetone solution was slowly poured into more stirred ether (300 $cm^3$), resulting in a sticky pinkish precipitate. This was collected by vacuum filtration and dried under vacuum overnight, resulting in a peach coloured powder (0.41 g). $^1H$ and $^{13}C$ nmr spectra of this material in $d_6$-dmso indicated a mixture of different species.

The sample was dissolved in $H_2O$ and adsorbed on an S-Sepharose column (3.5 x 11.0 cm). After washing with a small volume of water (20 $cm^3$) the adsorbed complexes were eluted with 1 M $NaClO_4$. A total of eight bands separated during elution. *Bands 1*, *2*, *3*, *5*, and *7* were present in trace amounts only, and all but *Band 1* were pink or mauve and so were discarded. *Band 1* was a small quantity of the condensed monomer **31**. *Band 8* was some pink material that remained strongly adsorbed to the column, even when the $Na^+$ concentration of the eluent was increased to 4 M. Forty percent of the starting material was recovered in *Band 4*. *Band 6* was a highly charged orange species. Excess $NaClO_4$ was removed from the eluent of this band by reducing its volume by rotary evaporation (without heating the water bath), and filtering the salt as it precipitated. More $NaClO_4$ was removed by taking the now concentrated solution and passing it down a Biogel column (3.0 x 15.0 cm) twice. Sufficient salt was removed to allow a $^{13}C$ spectrum to be obtained. Attempts to obtain a $^{59}Co$ spectrum and to recrystallise the complex for elemental microanalysis and for $^{1}H$ nmr spectrometry were unsuccessful; the complex continually degraded to the monomer. $^{13}C$ nmr ($D_2O/H_2O$): $\delta$ 184.7 (=C-COO), 179.8 (Co-COO), 165.2 (C=N), 60.5 ($C_q$), 39.9 ($CH_2$), 25.0 ($CH_3$).

***Synthesis of the binuclear complex formed by the self condensation of $[(en)_2Co(ala\text{-}im)]^{2+}$***

In a similar manner, equimolar quantities of the protonated and deprotonated complex, $[(en)_2Co(NHC(CH_3)COO)]^{2+}$ and $[(en)_2Co(NC(CH_3)COO)]^{+}$, were dissolved in dmf (10 $cm^3$). The resulting deep orange-brown solution was stirred at ~ 20 °C for 50 hours. After diluting with water (100 $cm^3$) a few drops of 3 M acetic acid were added until the pH of the solution was about 3. This solution was then chromatographed on a Dowex column (3.5 x 15.0 cm). After washing with water (~50 $cm^3$) and 0.5 M HCl, the complexes were eluted with 2 M HCl. Four bands separated and were reduced to dryness by rotary evaporation (without heating the water bath):

*Band 1:* Orange. Identified by $^{1}H$ nmr as the monomer, ~ 5%.

*Band 2:* Orange. Identified by $^{1}H$ nmr as the protonated starting material, ~ 50%.

*Band 3:* Pink.

*Band 4:* Orange. Its behaviour on the column indicated that it was highly charged. After reducing the eluent to dryness $^{13}C$ and $^{1}H$ nmr were acquired of a solution of the residue. These indicated that there were at least two cobalt centres, with a multiplicity of carboxyl, imine, en-$CH_2$

and methyl signals. The spectra are reproduced in Figure 13 for comparison with the isolated monomers.

*Synthesis of $[(en)_2Co(NH_2C(CH_3)(CH_2COCOOH)COO)]Cl_2$*

A sample of $[(en)_2Co(NHC(CH_3)COO)]Cl_2$ (3.00 g) was dissolved in a solution of carbonate buffer (5 cm$^3$, $[CO_3^{2-}] = [HCO_3^-] = 0.5$ M) in water (20 cm$^3$). Sodium pyruvate (4.90 g) was added to this solution and the resulting solution stirred at 25 $^oC$. The progress of the reaction was monitored by column chromatography. At 25, 35 and 45 minutes after the beginning of the reaction a couple of drops of the reaction mixture was adsorbed to a small Sephadex column (1.0 x 4.0 cm). At 25 minutes there was already an appreciable amount of a low-charge species (presumed to be the product) present. At 45 minutes no further increase in the quantity of this species was noted so the remainder of the reaction mixture was quenched by addition of 3 M acetic acid to pH 6. After diluting this solution with water to a total volume of 300 cm$^3$ the complexes were adsorbed to a Sephadex column (5.5 x 25.5 cm) and eluted with 0.25 M NaCl. Two major bands, both orange, separated. The first is the larger of the two. A little orange coloured material escaped at the very beginning of elution. This was presumed to be a small quantity of **32a**, the pendant carboxyl group deprotonated to leave the complex with a charge of $^+1$. The two large bands were desalted on small columns of Dowex, and the eluents reduced to dryness by rotary evaporation.

*Band 1:* The product, $[(en)_2CoNH_2C(CH_3)(CH_2COCOOH)(COO)]Cl_2$, was recrystallised from $H_2O$ by the addition of ethanol and refrigerating the resulting solution for 48 hours. The pale orange powder that precipitated was collected by vacuum filtration and washed with small quantities of ethanol and diethyl ether before drying over silica gel (1.55 g, 43%). Analysis calculated for $[CoC_{10}H_{24}N_5O_4Cl_2].H_2O$: Co, 13.83; C, 28.18; H, 6.15; N, 16.43; Cl, 16.64. Found: Co, 13.7;

C, 28.1; H, 6.2; N, 16.8; Cl, 16.8. $^1$H nmr (2 M DCl): $\delta$ 5.60 - 4.30 (br, 8H, en-$NH_2$), 3.32 (ABq, 2H, $CH_2$), 1.61 (s, 3H, $CH_3$). $^{13}$C nmr (2M DCl): $\delta$ 185.3 (Co-COO); 177.8, 173.3 (COO); 74.9 ($C_q$); 46.7, 46.1, 45.7, 45.0 (4 x en-$CH_2$); 44.4 ($CH_2$); 26.7 (CO-$CH_3$); 26.2 ($C_q$-$CH_3$).

*Band 2:* $[(en)_2CoNHC(CH_3)(COO)]Cl_2$.

***Further reaction of $[(en)_2Co(NH_2C(CH_3)(CH_2COCOOH)COO)]Cl_2$ in base***

A sample of $[(en)_2CoNH_2C(CH_3)(CH_2COCOOH)(COO)]Cl_2$ (0.15 g) was dissolved in carbonate buffer (10 cm$^3$, $[CO_3^{2-}] = [HCO_3^-] = 0.5$ M). The resulting solution was stirred at 25 $^o$C for 2 hours, during which time the solution darkened in colour. The reaction was quenched by diluting it to 50 cm$^3$ with water and adding 3 M acetic acid to give a final pH of 5. This solution was passed through a Sephadex column (3.0 x 15.0 cm). The adsorbed complexes were washed with $H_2O$ (pH 4 with 3 M acetic acid) before eluting with 0.5 M NaCl (pH 4 with 3 M acetic acid). Two orange bands formed and were collected separately. The solutions were desalted on small Dowex columns and the resulting eluents reduced to dryness by rotary evaporation. The recovered complexes were identified by $^1$H nmr spectrophotometry.

*Band 1:* The condensed product, **32a**, (0.02g, 13%). $^1$H and $^{13}$C nmr spectra are as quoted previously.

*Band 2:* $[(en)_2CoNHC(CH_3)(COO)]^{2+}$.

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# CHAPTER 5

## N-Alkylation of α-Imino Acids Coordinated to Co(III)

## Introduction

The previous chapter described the products of an important self condensation reaction which occurred in non aqueous basic conditions and in preference to alkylation of the imine-N of **2**, Figure 1. This process of self condensation is interesting and produces a complex containing an unusual new amino acid, **4**. However the N-alkylation reaction is useful for the synthesis of many new amino acids and needs to be examined more closely. For example, it is important to gauge the range of haloalkanes which react fast enough with the deprotonated imino acid so that **4** is no more than a minor by-product. In the experiments described below, changes were made to the two substrates of the alkylation reaction to determine under what circumstances a useful reaction occurred. Firstly, the alkylation reaction was performed with a range of haloalkanes, to determine which would react most effectively. Secondly, alkylation reactions were attempted using a number of tetraamine complexes, including $[(bipy)_2Co(ala\text{-}im)]^{2+}$. This last complex was chosen because the bipyridine co-ligands should not interfere with the subsequent synthesis of a heterocycle on the metal complex template.

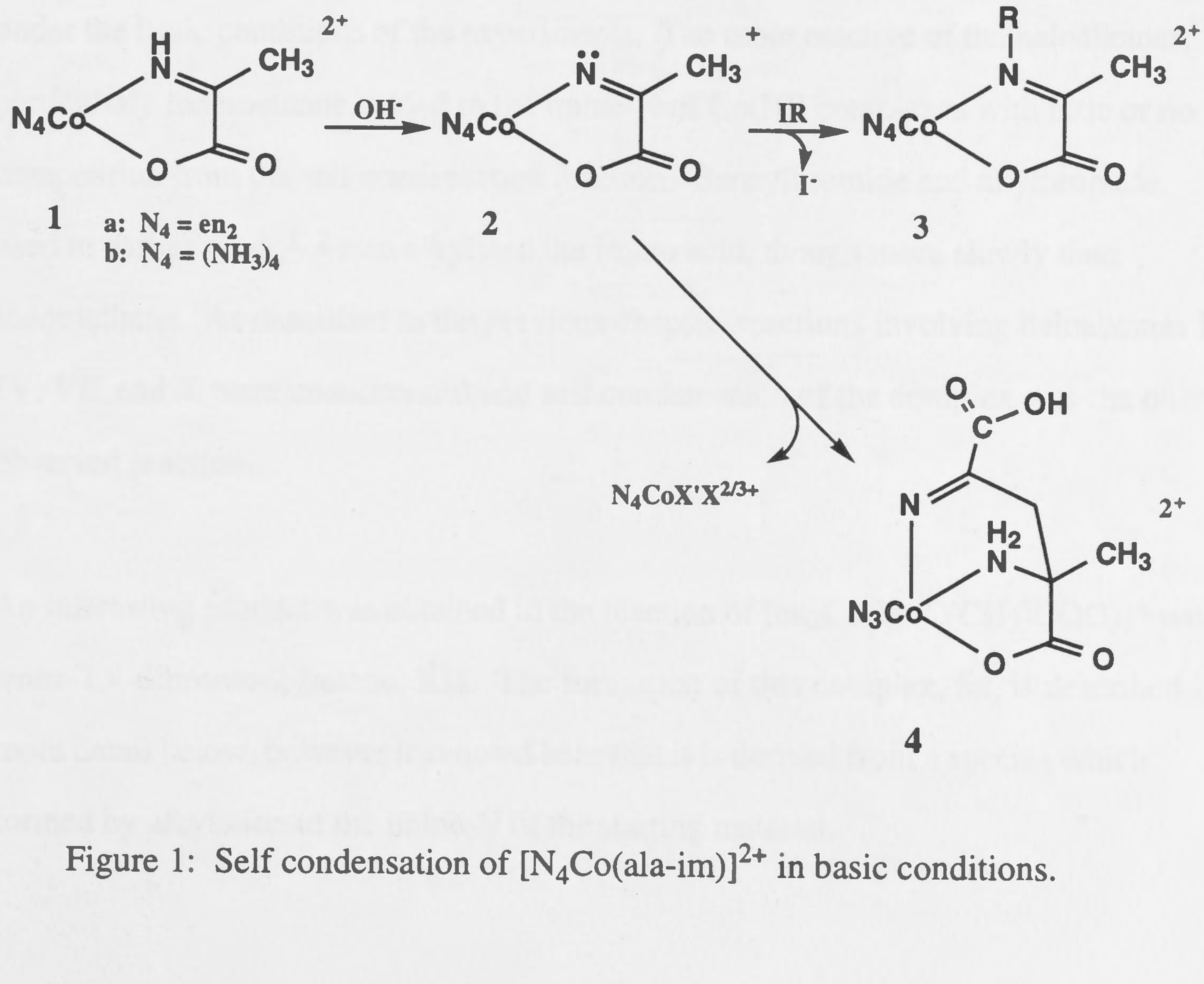



Figure 1: Self condensation of $[N_4Co(ala\text{-}im)]^{2+}$ in basic conditions.

## Results and Discussion

### *SYNTHESES*

#### *Reactions of complexes of the type $[N_4Co(N{=}C(R)COO)]^+$ with haloalkanes.*

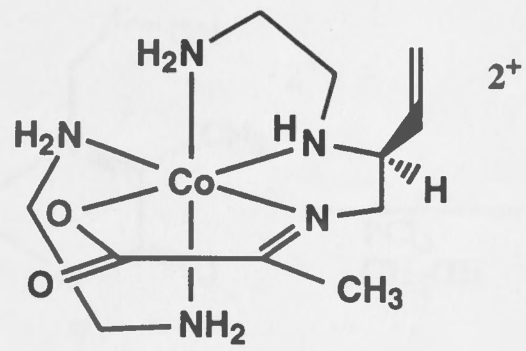
Table 1 details the reactions of a number of haloalkanes (**I - XII**) with some Co(III) imino acid complexes. The outcomes of these reactions are quite variable; in some instances the reaction proceeds rapidly to completion, in others the haloalkane does not react with the complex in the time frame of the experiment. In all but the most rapid of alkylation reactions self condensation of the Co(III) complex (described in the preceding chapter) is a competing reaction. A tetraammine complex of phenyl glycine imine was used in some reactions because there is no self condensation reaction to compete with the imine-N alkylation reaction. However, though previous experiments[3] have shown it to react with **I** and **VIII**, it did not react with haloalkanes such as **II**, **IV** and **V**. The bipy complex proved to be quite unstable and decomposed under the basic conditions of the experiments. The more reactive of the haloalkanes, particularly iodomethane, added to the imine-N of Co(III) complexes with little or no competition from the self condensation reaction. Benzylbromide and allylbromide, used in earlier work,[1, 2] also alkylated the imino acid, though more slowly than iodomethane. As described in the previous chapter, reactions involving haloalkanes **II**, **IV**, **VII** and **X** were unsuccessful and self condensation of the complex was the only observed reaction.

An interesting product was obtained in the reaction of $[en_2Co(N{=}C(CH_3)COO)]^+$ with *trans*-1,4-dibromo-2-butene, **XII**. The formation of this complex, **5a**, is described in more detail below, however it is noted here that it is derived from a species which formed by alkylation of the imine-N of the starting material.

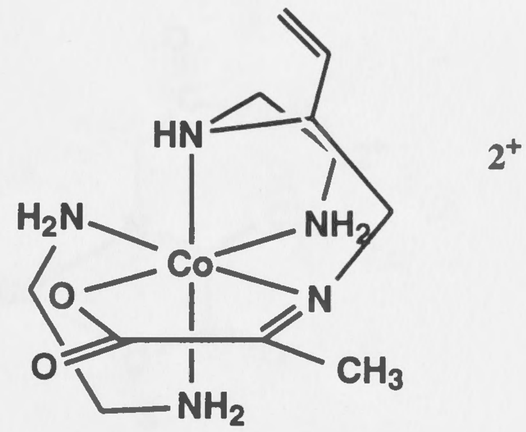
***Table 1: Imine-N Alkylation of α-Imino Acids Chelated to Co(III)***

| Haloalkane | $N_4$: | $(NH_3)_4$ | $(en)_2$ | $(bipy)_2$ | $(NH_3)_4$ |
|---|---|---|---|---|---|
| | imino acid: | ala-im | ala-im | ala-im | phenyl-gly-im |
| $CH_3I$ | **I** | Yes[a] | Yes[b] | Yes[c] | Yes[b] |
| $CH_2I_2$ | **II** | No[c] | No[c] | - | No[c] |
| $ICH_2CH_3$ | **III** | Yes[c] | - | - | - |
| $ICH_2CH_2I$ | **IV** | No[c] | No[c] | - | No[c] |
| $ICH_2CH_2OH$ | **V** | No[c] | No[c] | | No[c] |
| $ICH_2COO^-Na^+$ | **VI** | Yes[c] | - | - | - |
| $BrCH_2CH_2Br$ | **VII** | No[c] | No[c] | - | - |
| $BrCH_2CH{=}CH_2$ | **VIII** | Yes[a] | Yes[b] | - | Yes[b] |
| $BrCH_2C_6H_5$ | **IX** | Yes[a] | Yes[a] | - | - |
| $ICH_2CH_2CH_2I$ | **X** | No[c] | No[c] | - | - |
| $BrCH_2CH_2CH_2Cl$ | **XI** | No[c] | No[c] | - | - |
| $BrCH_2CH{=}CHCH_2Br$ | **XII** | - | Yes[c] | No[c] | - |

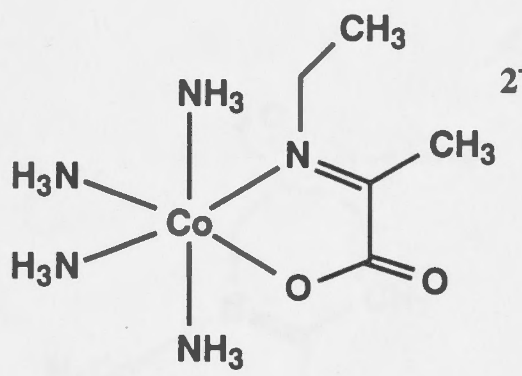
[a]Harrowfield and Sargeson, *J. Am. Chem. Soc.*, 1979, **101**, 1514. [b] Drok *et. al.*, *Aust. J. Chem.*, 1993, **46**, 1557. [c]This work.



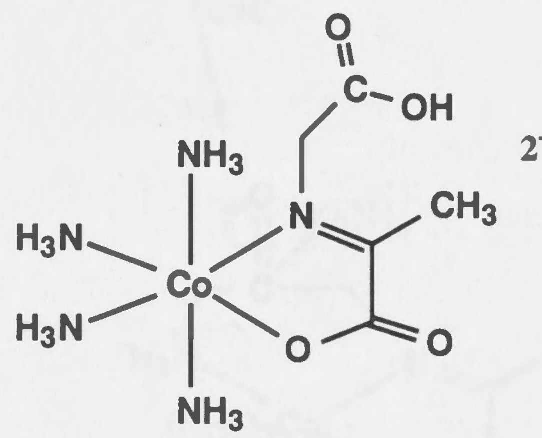
**5a**



**5b**



**6**



**7**

Reactions involving alkanes bearing two different functional groups produced mixed results. No alkylation was observed in reactions of $[(NH_3)_4Co(N{=}C(CH_3)COO)]^+$ and $[(en)_2Co(N{=}C(CH_3)COO)]^+$ with 2-iodoethanol and 1-bromo-3-chloropropane (**V** and **XI**). By contrast, reaction of $[(NH_3)_4Co(N{=}C(CH_3)COO)]^+$ with iodoethane and sodium iodoacetate (**III** and **VI**) resulted in the imine-N alkylated products, **6** and **7**, in yields of 48% and 66% respectively. It might be possible to synthesise the ethyl ester of **7**, and thereby synthesise **10**, 2-carboxy-4-hydroxy-pyrrole, from this complex, Figure 2. However, in view of previous experience, it is most probable that one of two reactions will occur instead:

i) the ester would hydrolyse under basic conditions before cyclisation occurred.

ii) one of the ammonia ligands deprotonates and the subsequent condensation reaction results in a new amino acid amido species **12**.

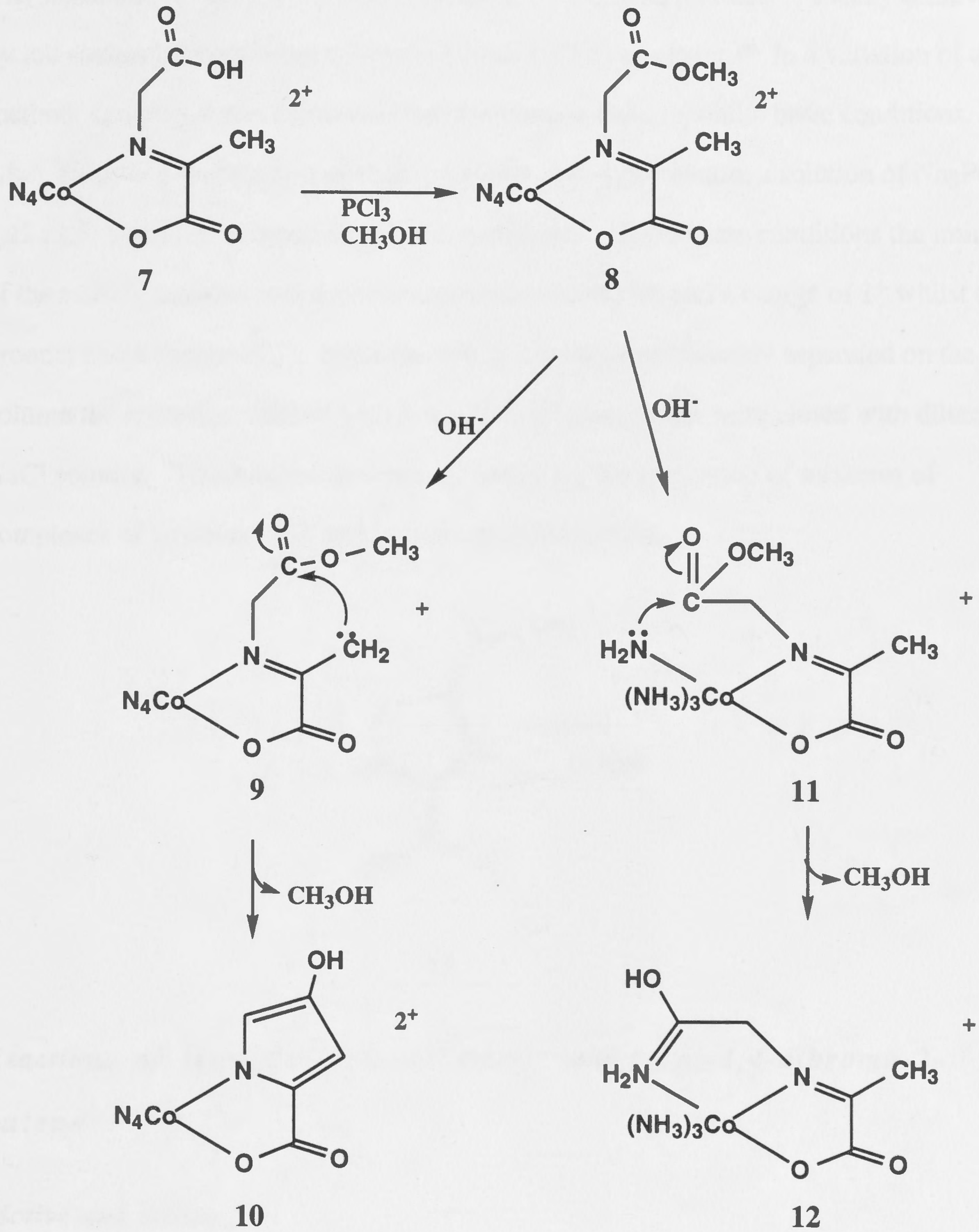
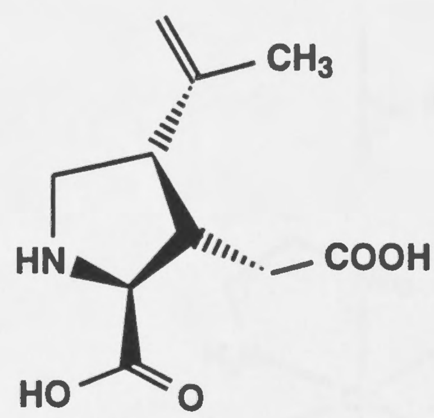


Figure 2: Both coordinated amido ion and deprotonated β-methyl moieties are capable of reacting with the pendant ester of **8**.

Because of these considerations no attempts were made to esterify the pendant acetic acid moiety of **7**. However, reduction of the new imino acids **6** and **7** described here would lead to interesting examples of novel amino acids.

The separation of starting material from imine-N alkylated products is usually achieved by ion exchange chromatography using dilute HCl as an eluent.[1b] In a variation of this method, complex **6** was separated from the starting material under basic conditions. After adsorbing the reaction mixture on an ion exchange column, a solution of $Na_3PO_4$ (pH 12.3) was used to begin eluting the complexes. Under these conditions the imine of the starting material was deprotonated and the complex had a charge of $1^+$ whilst the product had a charge of $2^+$. Once the two species were sufficiently separated on the column the resin was washed with water and both complexes were eluted with dilute NaCl solution. This method also proved useful for the separation of mixtures of complexes of an amino acid and its analogous imino acid.




**13**

## ***Reactions of $[(en)_2Co(N{=}C(R)COO)]^+$ with trans-1,4-dibromo-2-butene***

*Motive and Strategy*

Kainic acid, **13**, is a heterocyclic amino acid that has been isolated from fungi.[3a] It has attracted interest because of its neuroexcitant properties and its anthelmintic and insecticidal activities.[3b] A complex derivative of proline, it presents a synthetic challenge for the chemistry made available by imino acid complexes of Co(III). A feasible strategy by which a chelated imino acid could react with a haloalkane such as *trans*-1,4-dibromo-2-butene to produce analogues of the naturally occurring amino acid is described in Figure 3. In a procedure similar to that proposed for the synthesis of other heterocyclic amino acids (see Chapter 2), *trans*-1,4-dibromo-2-butene would be

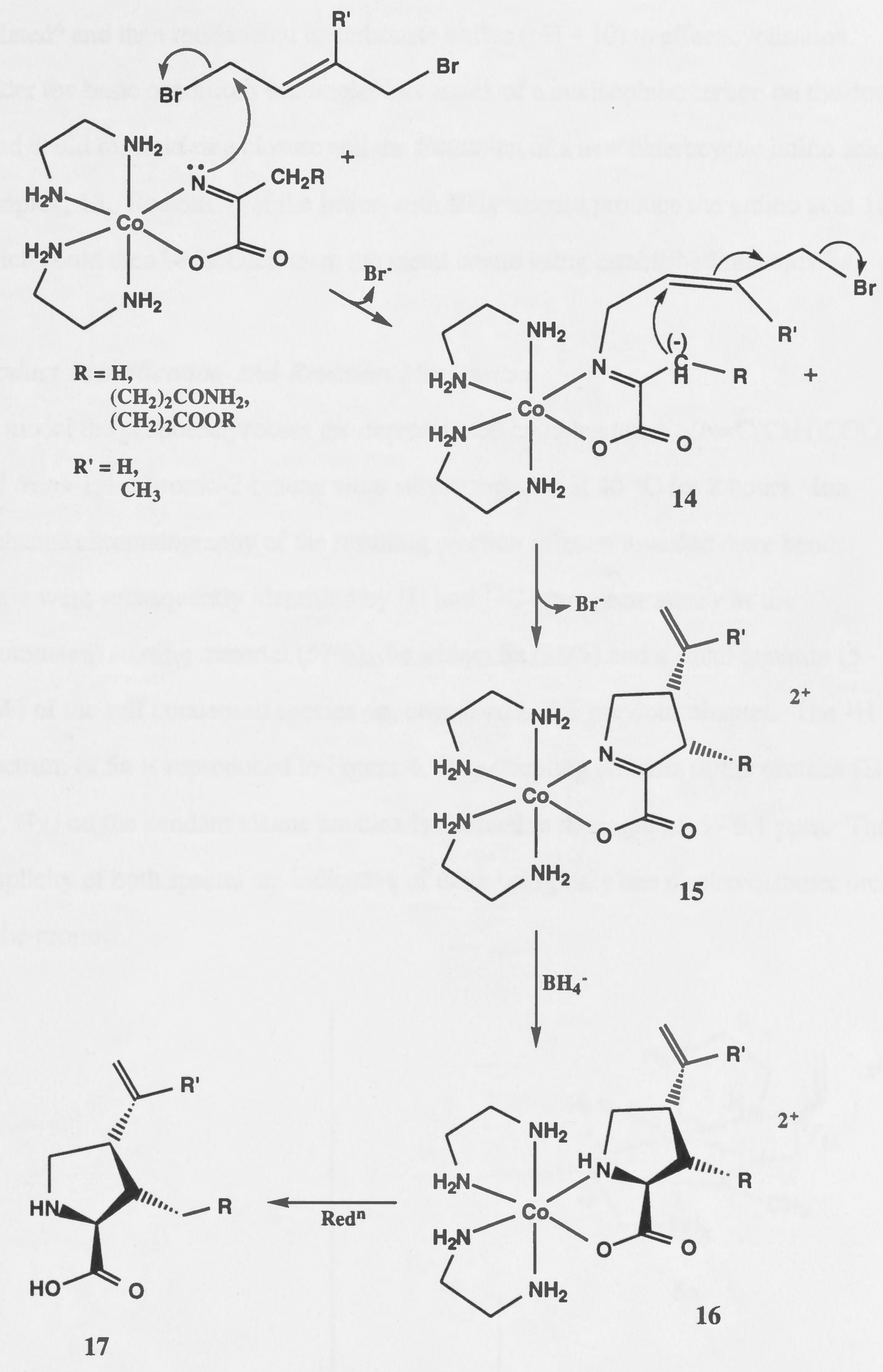
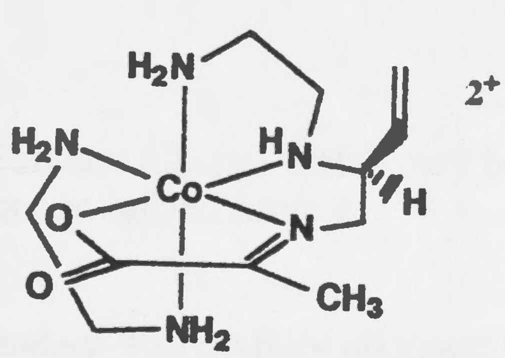



Figure 3: Proposed synthesis of kainic acid analogues.

added to the imine-N deprotonated complex, forming **14**. This complex could be isolated[4] and then redissolved in carbonate buffer (pH ~ 10) to effect cyclisation. Under the basic conditions intramolecular attack of a nucleophilic carbon on the double bond could result in ring closure and the formation of a new heterocyclic imino acid complex, **15**. Reduction of the imine with $BH_4^-$ should produce the amino acid **16**, which could then be isolated from the metal centre using established methods.[5]

*Product Identification and Reaction Mechanism*

To model the proposed process the deprotonated complex $[en_2Co(N{=}C(CH_3)COO)]^+$ and *trans*-1,4-dibromo-2-butene were stirred together at 40 °C for 2 hours. Ion exchange chromatography of the resulting reaction mixture revealed three bands. These were subsequently identified by $^1H$ and $^{13}C$ nmr spectrometry as the (protonated) starting material (57%), the adduct **5a** (28%) and a small quantity (5 - 10%) of the self condensed species **4a**, described in the previous chapter. The $^1H$ nmr spectrum of **5a** is reproduced in Figure 4. The coupling patterns of the protons ($H_A$, $H_B$, $H_X$) on the pendant alkene are clearly defined in the region 5.6 - 6.1 ppm. The simplicity of both spectra are indicative of there being only one diastereoisomer present in the product.




**5a**

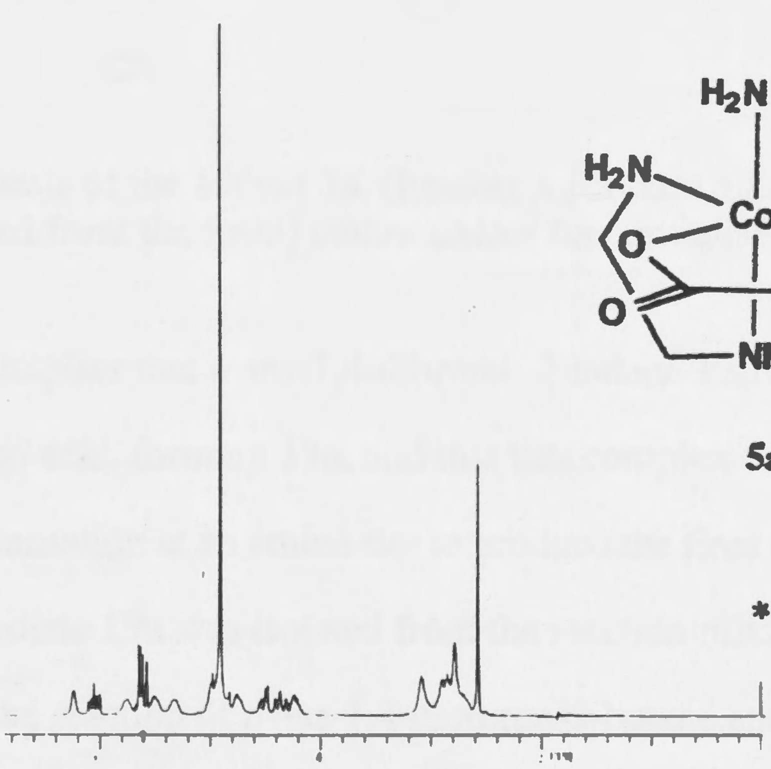



Figure 4: $^1H$ nmr spectrum of **5a** (0.1 M DCl, *NaTPS as internal standard).

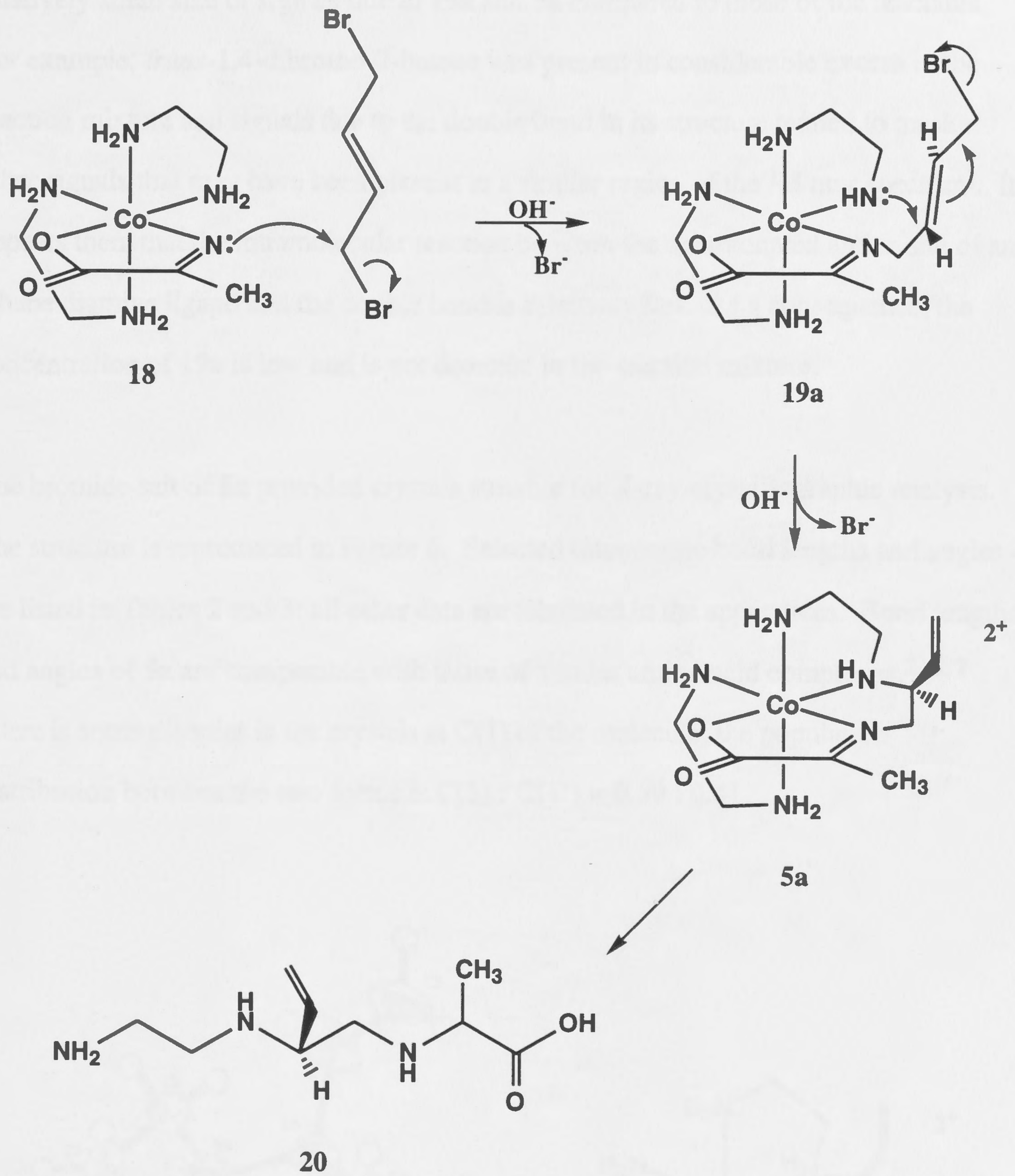
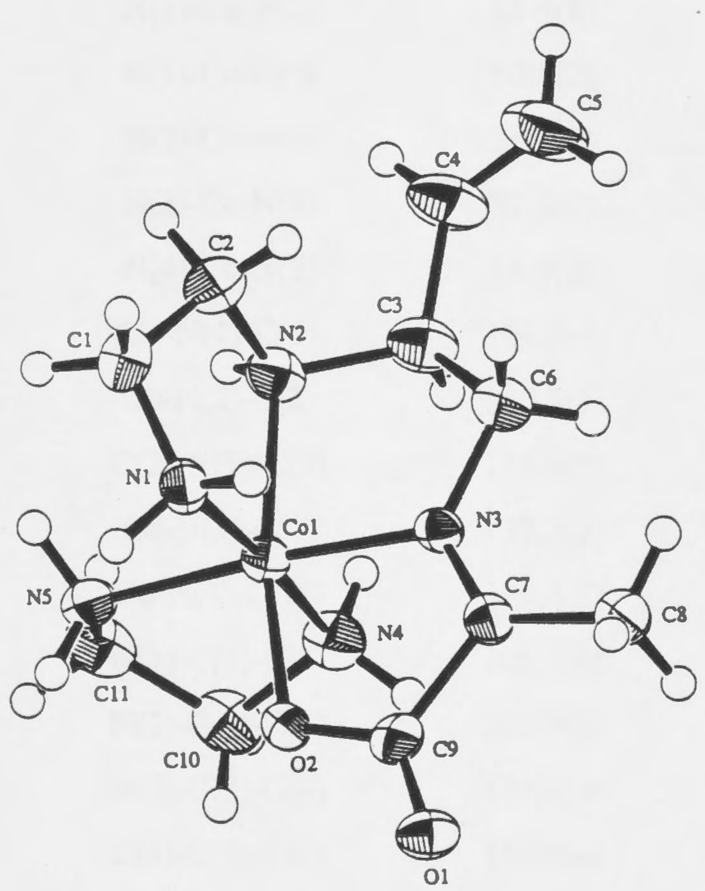
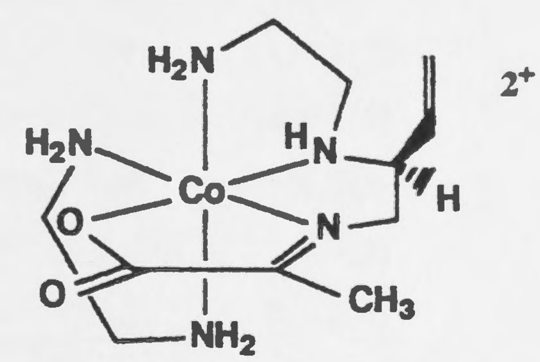



Figure 5: Synthesis of the adduct 5a, (bearing a pendant alkene) which may be isolated from the metal centre and/or functionalised further.

The isolation of **5a** implies that *trans*-1,4-dibromo-2-butene successfully alkylates the imine-N of the imino acid, forming **19a**, and that this complex then undergoes an intramolecular condensation at an amine site to produce the final product, Figure 5. None of the intermediate **19a** was isolated from the reaction mixtures. In order to detect its presence the reaction of *trans*-1,4-dibromo-2-butene and $[(en)_2Co(N{=}C(CH_3)COO)]^+$ in $d_6$-dmso was monitored by $^1H$ nmr spectrometry. However, no signals attributable to **19a** were discernible. This was due, in part, to the relatively small size of signals due to **19a** and **5a** compared to those of the reactants. For example, *trans*-1,4-dibromo-2-butene was present in considerable excess in the reaction mixture and signals due to the double bond in its structure tended to mask other signals that may have been present in a similar region of the $^{1}H$ nmr spectrum. It appears then, that the intramolecular reaction between the deprotonated amine site of an ethane diamine ligand and the double bond is relatively fast. As a consequence, the concentration of **19a** is low and is not detected in the reaction mixture.

The bromide salt of **5a** provided crystals suitable for X-ray crystallographic analysis. The structure is reproduced in Figure 6. Selected interatomic bond lengths and angles are listed in Tables 2 and 3; all other data are tabulated in the appendices. Bond lengths and angles of **5a** are comparable with those of similar amino acid complexes.[2, 6, 7] There is some disorder in the crystals at C(1) of the molecule; the population distribution between the two forms is C(1) : C(1') = 0.59 : 0.41.




**5a**

Figure 6: ORTEP diagram of the cation **5a** of the crystal structure. Ellipsoids show 50% probability levels and hydrogen atoms have been drawn as circles of arbitrary small radius.

***Table 2: Interatomic Distances (Å) for Non-Hydrogen Atoms of 5a.***

| | | | | | |
|---|---|---|---|---|---|
| Co-O(2) | 1.921(3) | N(1)-C(1) | 1.419(10) | C(1)-C(1') | 0.84(1) |
| Co-N(1) | 1.938(4) | N(1)-C(1') | 1.55(1) | C(1)-C(2) | 1.56(1) |
| Co-N(2) | 1.964(4) | N(2)-C(2) | 1.487(6) | C(1')-C(2) | 1.27(2) |
| Co-N(3) | 1.877(3) | N(2)-C(3) | 1.522(6) | C(3)-C(4) | 1.497(6) |
| Co-N(4) | 1.965(4) | N(3)-C(6) | 1.465(5) | C(3)-C(6) | 1.528(6) |
| Co-N(5) | 1.962(4) | N(3)-C(7) | 1.266(5) | C(4)-C(5) | 1.296(7) |
| O(1)-C(9) | 1.219(5) | N(4)-C(10) | 1.484(6) | C(7)-C(8) | 1.49(6) |
| O(2)-C(9) | 1.295(5) | N(5)-C(11) | 1.487(6) | C(7)-C(9) | 1.529(6) |
| | | | | C(10)-C(11) | 1.496(7) |

***Table 3: Interatomic Angles (°) Involving Non-Hydrogen Atoms of 5a.***

| | | | |
|---|---|---|---|
| O(2)-Co-N(1) | 89.8(1) | O(2)-Co-N(2) | 166.8(1) |
| O(2)-Co-N(3) | 83.0(1) | O(2)-Co-N(4) | 89.1(1) |
| O(2)-Co-N(5) | 93.8(1) | N(1)-Co-N(2) | 85.4(2) |
| N(1)-Co-N(3) | 92.6(1) | N(1)-Co-N(4) | 175.1(2) |
| N(1)-Co-N(5) | 90.4(2) | N(2)-Co-N(3) | 84.9(1) |
| N(2)-Co-N(4) | 96.7(2) | N(2)-Co-N(5) | 98.5(2) |
| N(3)-Co-N(4) | 91.9(2) | N(3)-Co-N(5) | 175.6(2) |
| N(4)-Co-N(5) | 84.9(2) | Co-O(2)-C(9) | 114.1(3) |
| Co-N(1)-C(1) | 114.2(4) | Co-N(1)-C(1') | 107.2(6) |
| Co-N(2)-C(2) | 110.3(3) | Co-N(2)-C(3) | 106.9(3) |
| C(2)-N(2)-C(3) | 112.8(4) | Co-N(3)-C(6) | 116.7(3) |
| Co-N(3)-C(7) | 117.3(3) | C(6)-N(3)-C(7) | 126.0(4) |
| Co-N(4)-C(10) | 109.4(3) | Co-N(5)-C(11) | 110.5(3) |
| N(1)-C(1)-C(2) | 106.6(6) | N(1)-C(1')-C(2) | 115(1) |
| N(2)-C(2)-C(1) | 112.3(5) | N(2)-C(2)-C(1') | 114.1(8) |
| N(2)-C(3)-C(4) | 112.8(4) | N(2)-C(3)-C(6) | 107.3(4) |
| C(4)-C(3)-C(6) | 115.8(4) | C(3)-C(4)-C(5) | 126.6(5) |
| N(3)-C(6)-C(3) | 107.6(4) | N(3)-C(7)-C(8) | 127.6(4) |
| N(3)-C(7)-C(9) | 112.3(4) | C(8)-C(7)-C(9) | 120.1(4) |
| O(1)-C(9)-O(2) | 124.9(4) | O(1)-C(9)-C(7) | 122.0(4) |
| O(2)-C(9)-C(7) | 113.1(4) | N(4)-C(10)-C(11) | 107.1(4) |
| N(5)-C(11)-C(10) | 107.2(4) | | |

Depending on the coligand amine (N(**1**) or N(**2**)) that is involved in the second addition-elimination reaction with the alkene one of two isomers, **5a** or **5b**, may form.[7] In isomer **5a**, the new polyamine chain O(2)-C(9)-C(7)-N(3)-C(6)-C(3)-N(**2**)- wraps around the Co(III) center and is described as the *meridional*, or *mer*, isomer. In isomer **5b**, the chain O(2)-C(9)-C(7)-N(3)-C(6)-C(3)-N(**1**)- occupies an octahedral face and is the facial, or *fac*, isomer.

The crystal structure of the product demonstrates that the addition-elimination reaction between $[(en)_2Co(ala\text{-}im)]^{2+}$ and *trans* -1, 4 - dibromo-2-butene is regioselective, the *mer* isomer **5a** being the only product. Previous studies of reactions between pendant groups on amino- and imino- acid chelates and amido ions on the coligands have found that the *mer* isomer is favoured over the *fac* isomer. An examination of Dreiding models demonstrated that this is most likely to be because deprotonated N(**2**) is orientated in such a way that the lone pair may react more easily with the alkene than can the lone pair on deprotonated N(**1**).

The addition-elimination reaction generates a new quaternary carbon at C(**3**), with the accompanying prospect of stereoselectivity of the reaction. The isomer which forms will be dependent on whether the alkene pendant arm is orientated 'pointing up' or 'pointing down' in the transition state. Figure 7 illustrates the rotamers of **19** which give rise to the complexes **5a** and **21**. In rotomer **19a** the lone pair of electrons on N(**2**) and carbon centre C(3) of the alkene are ideally aligned for reaction to take place, resulting in the complex **5a**, which has been isolated. By comparison, in rotamer **19b** the alkene group has swung out of alignment with the lone pair of N(**1**); steric interactions between the arm and the coligands mean that **21** does not form. The addition-elimination reaction between the pendent arm is thus both stereoselective and regioselective.

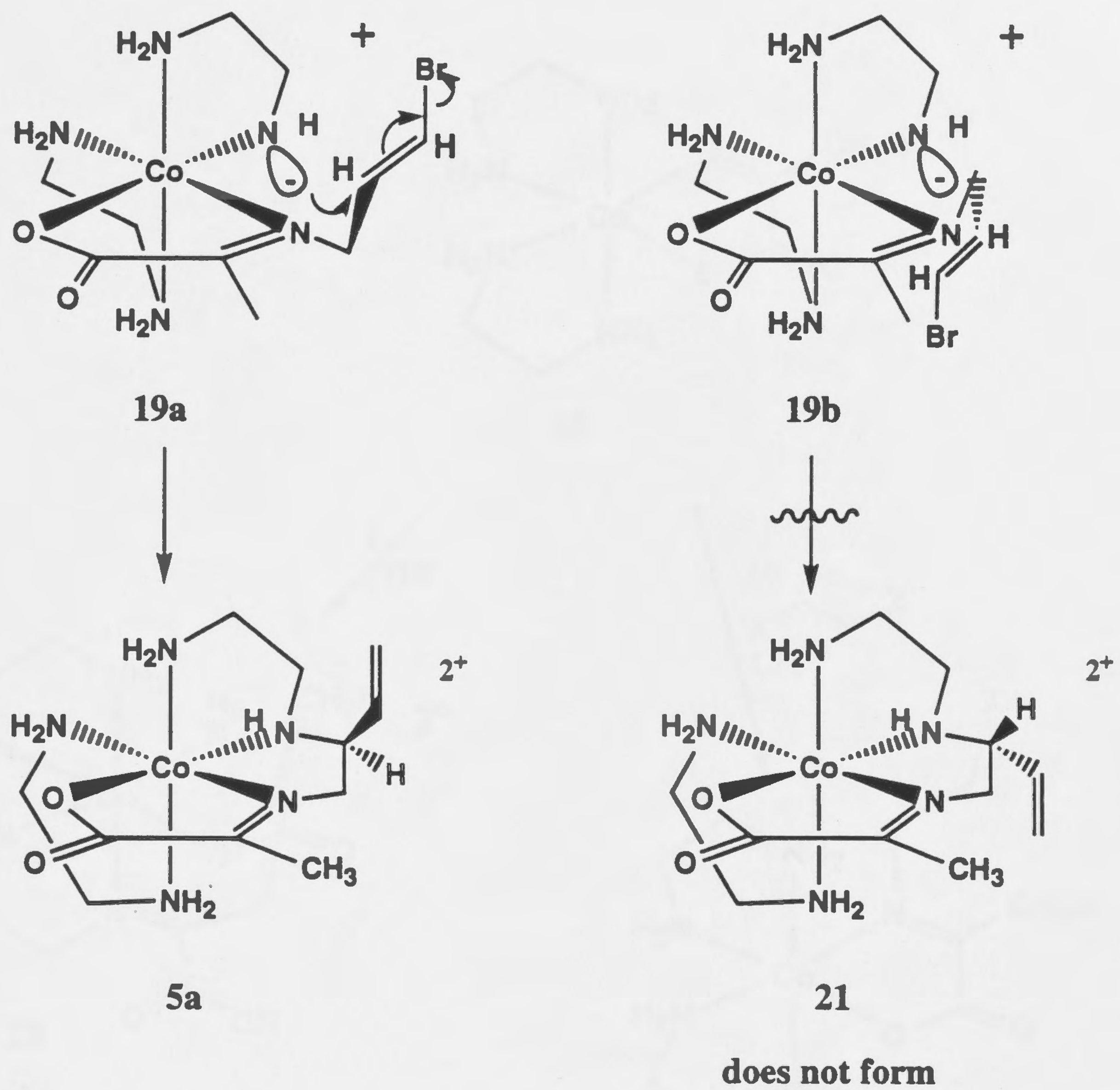



Figure 7: The intermediate, **19**, in the reaction of $[(en)_2Co(ala\text{-}im)]^{2+}$ with *trans* -1, 4,-dibromo-2-butene. Only **19a** can undergo a second addition-elimination reaction, to form the product **5a**. Steric interactions between the coligand and the pendent arm prevent reaction to form **21**.

The condensation reaction between an amine of the coligands and the double bond on the pendant arm of the imino acid has proven remarkably facile. This type of reaction, whilst it leads to interesting products, is likely to obviate other syntheses of heterocyclic amino acids whch use a similar strategy, Figure 8. The $pK_a$ of the amine sites of the coligand, reduced on coordination to the metal centre, are typically 15 - 16 and thus are often less than those of carbon acids such as the β-methyl of the imino acid ligand. The reactivity of coordinated amines in basic conditions has been exploited in a number of previous syntheses. The first chelated imino acid complex was generated by intramolecular reaction of amido ion with coordinated pyruvate in basic solution.[8] Other publications have described Schiff base condensations between coordinated

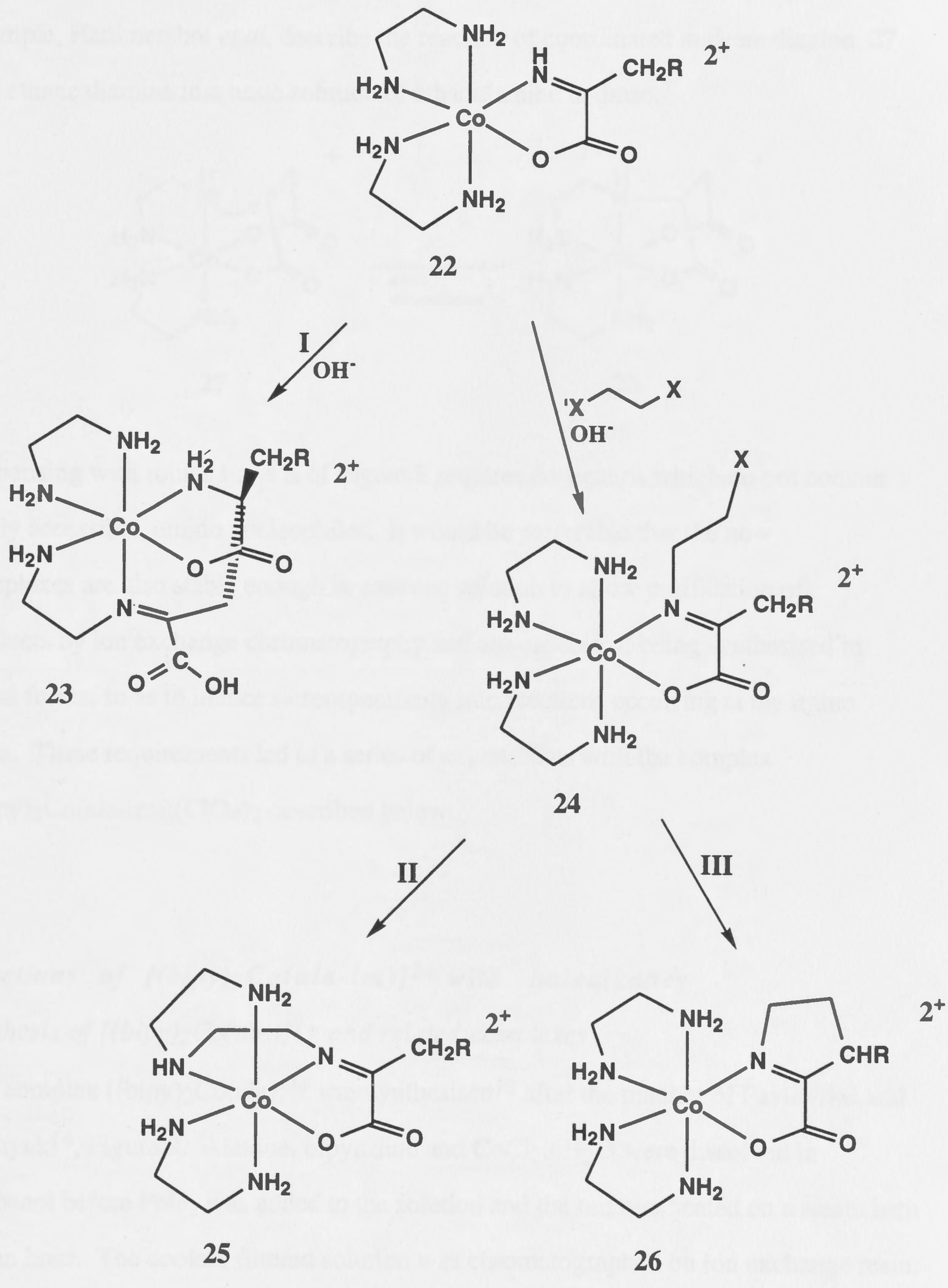
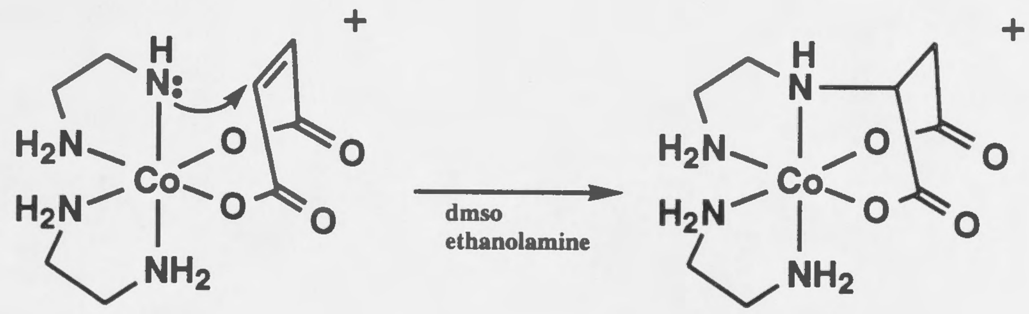



Figure 8: Reactions which compete with the synthesis of heterocycles from α-imino acidato complexes and dihaloalkanes (Reaction **III**). Reaction **I**: self condensation of the imino acid. Reaction **II**: addition-elimination reaction between an amido ion from the coligand and the pendant arm.

amines and various aldehydes,[2,9,10] nitriles[5,11] and alkenes[7] in basic conditions. For example, Hammershoi *et al.* describe the reaction of coordinated maleate dianion, **27** and ethane diamine in a basic solution of ethanolamine in dmso.[7]




**27** **28**

Dispensing with routes I and II of Figure 8 requires co-ligands which do not contain easily accessible, amido nucleophiles. It would be preferable that the new complexes are also stable enough in aqueous solution to allow purification of products by ion exchange chromatography and are capable of being synthesised in chiral forms, so as to induce stereospecificity into reactions occurring at the imino acids. These requirements led to a series of experiments with the complex $[(bipy)_2Co(ala\text{-}im)](ClO_4)_2$ described below.

### *Reactions of $[(bipy)_2Co(ala\text{-}im)]^{2+}$ with haloalkanes*

*Synthesis of $[(bipy)_2Co(ala)]^{2+}$ and related complexes*

The complex $[(bipy)_2Co(ala)]^{2+}$ was synthesised[13] after the manner of Pavlovskii and Poznyak[14], Figure 9. Alanine, bipyridine and $CoCl_2.6H_2O$ were dissolved in methanol before $PbO_2$ was added to the solution and the mixture heated on a steam bath for an hour. The cooled, filtered solution was chromatographed on ion exchange resin, producing just two bands ($[(bipy)_2Co(ala)]^{2+}$, **29**, and $[(bipy)_3Co]^{3+}$) and a quantity of brown material which remained strongly adsorbed to the top of the ion exchange column. The amino acid was then oxidised to the imino acid, **30** using thionyl chloride.[10] The imino acid complex was also converted to the corresponding triflate salt, $[(bipy)_2Co(ala\text{-}im)](CF_3SO_3)_2$, using anhydrous triflic acid.[16]

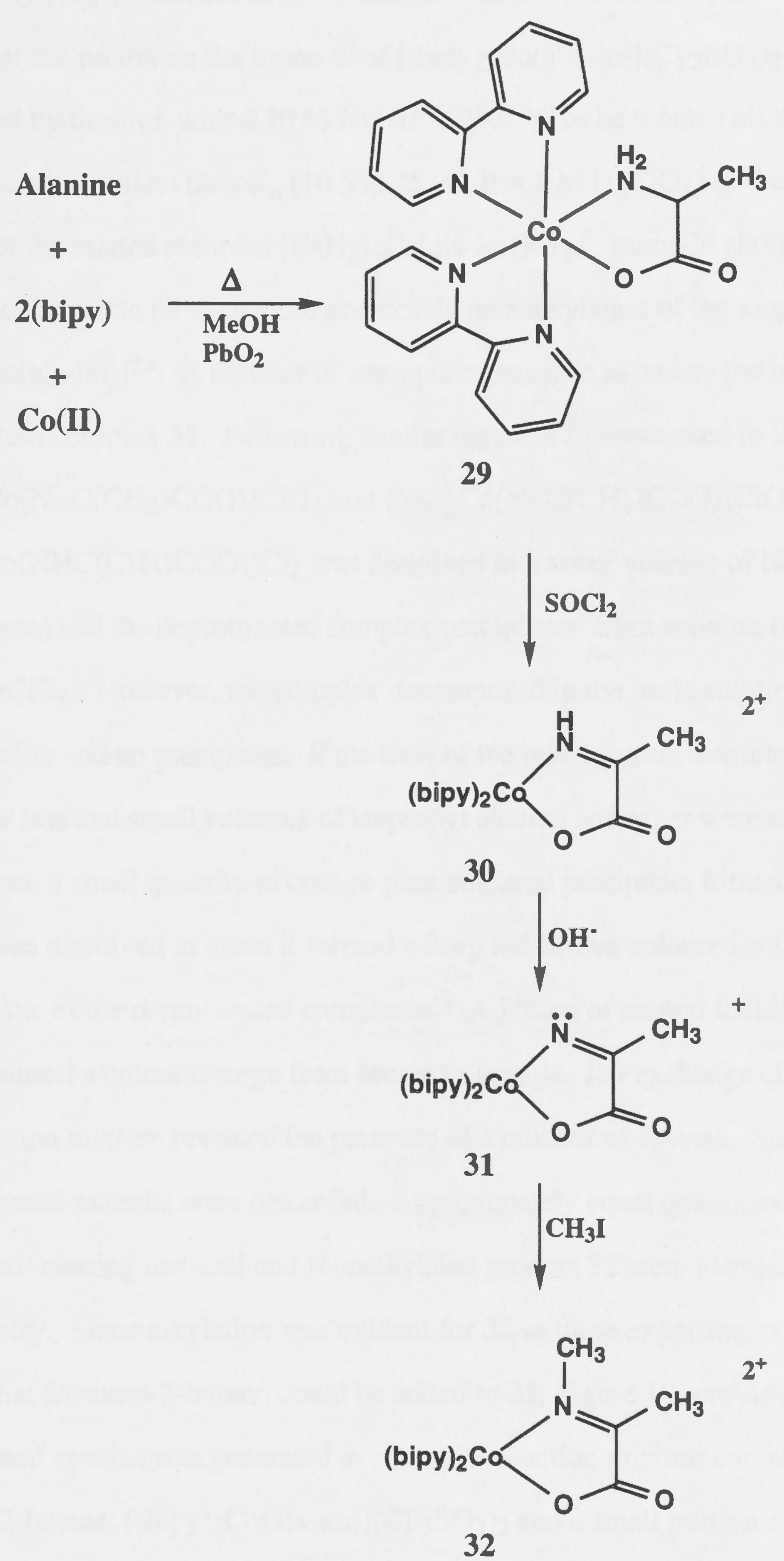



Figure 9: Synthesis of $[(bipy)_2Co(ala\text{-}im)]^{2+}$.[13]

*Synthesis of $[(bipy)_2Co(ala\text{-}im)]^{2+}$, and its reactions with haloalkanes*

The pKa of the proton on the imine-N of $[(bipy)_2Co(ala\text{-}im)](CF_3SO_3)_2$ was determined by titration with 0.10 M NaOH at 25.0 °C to be 9.58. This was significantly lower than the $pK_a$ (10.5 at 25 °C, $\mu$ = 1 M $NaClO_4$) of the proton on the imine-N of the related complex $[(NH_3)_4Co(ala\text{-}im)]Cl_2$.[8] Imine-N alkylation of **30** would be expected to be even more practicable than alkylation of the same site in $[(NH_3)_4Co(ala\text{-}im)]^{2+}$. A number of attempts were made to isolate the imine-N deprotonated complex **31**. Following similar methods to those used to isolate $[(NH_3)_4Co(N{=}C(CH_3)COO)]ClO_4$ and $[(en)_2Co(N{=}C(CH_3)COO)]ClO_4$, a sample of $[(bipy)_2Co(NHC(CH_3)COO)]Cl_2$ was dissolved in a small volume of NaOH (1.1 molar excess) and the deprotonated complex precipitated from solution by addition of excess $NaClO_4$. However, the complex decomposed in the basic solution, producing a black solution and no precipitate. If the time of the reaction was restricted to 20 seconds or less and small volumes of isopropyl alcohol and ether were added to the solution then a small quantity of orange-pink coloured precipitate formed. When this material was dissolved in dmso it formed a deep red-brown coloured solution characteristic of the deprotonated complexes.[8] Addition of methyl iodide to this solution caused a colour change from brown to orange. Ion exchange chromatography of the reaction mixture revealed the presence of a number of species. Small traces of green coloured material were discarded. Approximately equal quantities of the (protonated) starting material and N-methylated product **32** were identified by nmr spectrometry. Since alkylation was evident for **32** in these experiments, it was feasible that dibromo-2-butene could be added to **31**, Figure 10, provided the deprotonated species was generated *in situ*. The reaction mixture contained *trans*-1,4-dibromo-2-butene, $[(bipy)_2Co(ala\text{-}im)](CF_3SO_3)_2$ and a small portion of diisopropylethylamine in dmf. After quenching the reaction mixture with acetic acid the complexes in the reaction mixture were separated by ion exhange chromatography and identified by $^1H$ nmr spectrometry. Most of the material proved to be products resulting from the decomposition of the bipy complex in the basic conditions. There was also a small quantity of material whose nmr spectrum and behaviour on the column

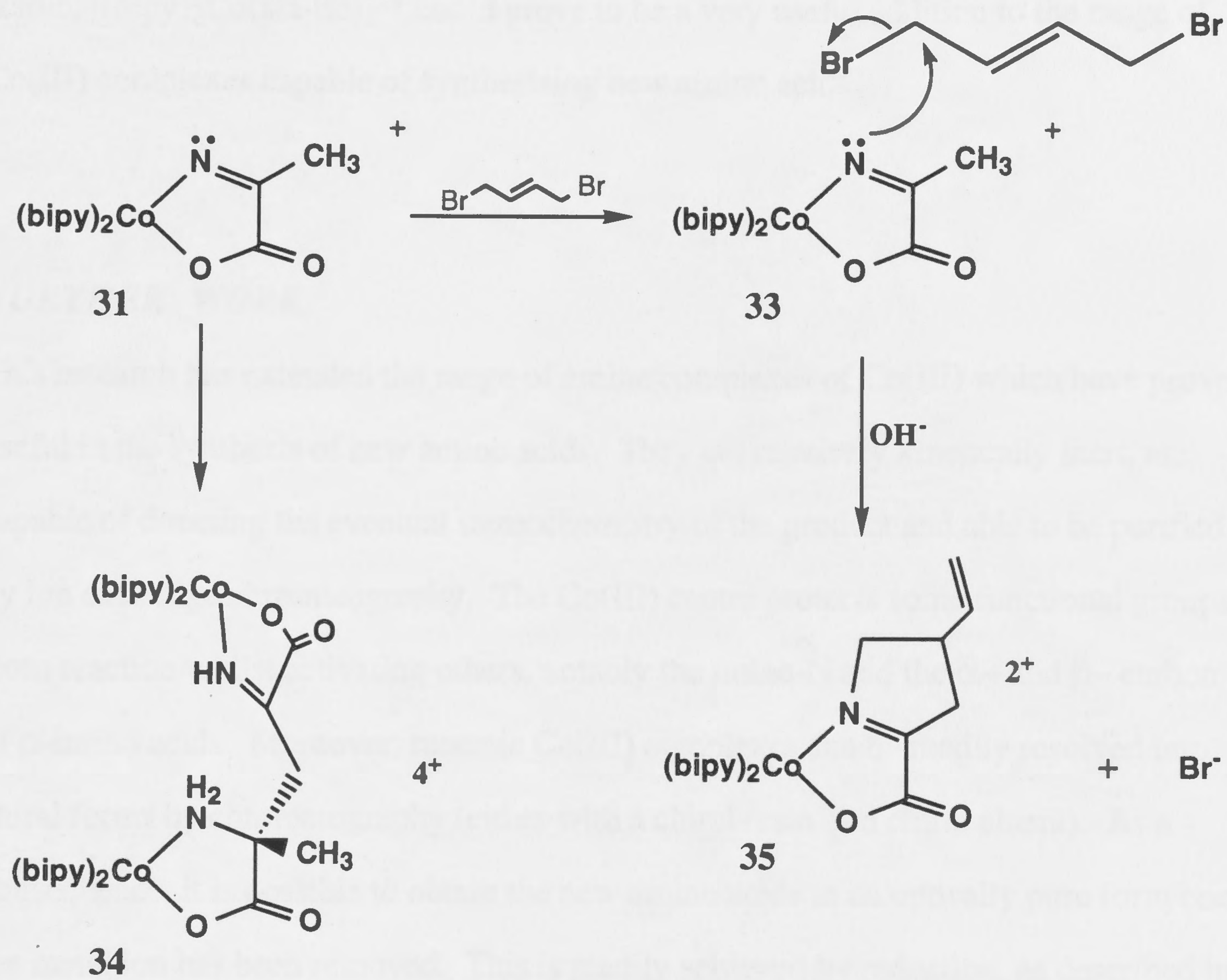



Figure 10: Proposed reaction of $[(bipy)_2Co(ala\text{-}im)]^{2+}$ with 1, 4 -dibromobutene, and binuclear byproduct.

implied that it was an analogue of the binuclear complexes described in Chapter 2 (**34**). None of the desired complex (**35**) was isolated from the reaction mixture.

*Conclusion*

The synthesis of $[(bipy)_2Co(N(CH_3)C(CH_3)COO)]^{2+}$ proves that the imine-N of $[(bipy)_2Co(ala\text{-}im)]^{2+}$ may be alkylated. This particular reaction must be qute swift to compete with decomposition. In other instances, such as the alkylation of $[(bipy)_2Co(ala\text{-}im)]^{2+}$ with *trans* -1, 4- dibromo-2-butene, the complex is not redox stable enough under the basic conditions for the desired reaction to be competetive. By contrast, the complex has proved quite stable under acidic conditions. For example, the amino acid was readily oxidised to the imino acid with thionyl chloride. These complexes may also be chromatographed on ion exchange resins with dilute HCl and the triflate salt was isolated from anhydrous triflic acid. Provided the conditions were acidic, $[(bipy)_2Co(ala\text{-}im)]^{2+}$ could prove to be a very useful addition to the range of Co(III) complexes capable of synthesising new amino acids.

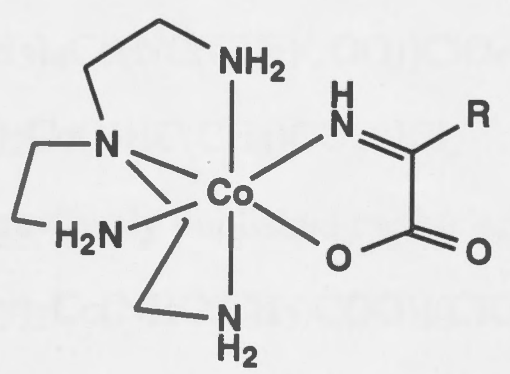
## *FURTHER WORK*

This research has extended the range of amine complexes of Co(III) which have proven useful in the synthesis of new amino acids. They are relatively kinetically inert, are capable of directing the eventual stereochemistry of the product and able to be purified by ion exchange chromatography. The Co(III) centre protects some functional groups from reaction whilst activating others, notably the imine-N and the α– and β– carbon of α-imino acids. Moreover, racemic Co(III) complexes can be readily resolved into chiral forms by chromatography (either with a chiral resin or a chiral eluent). As a consequence, it is possible to obtain the new amino acids in an optically pure form once the metal ion has been removed. This is readily achieved by reduction, as described in Chapter 3 of this thesis. Cobalt(III) complexes therefore, not only alter the regular organic reactivity, they offer simple new ways of doing organic syntheses.

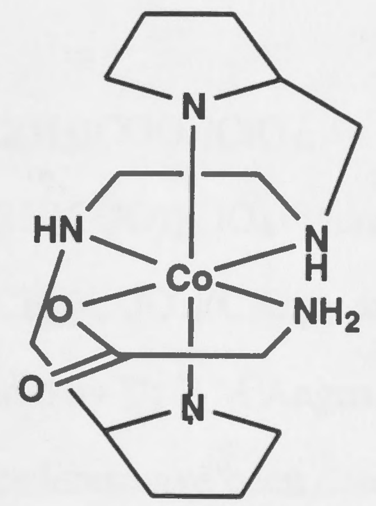
Cobalt(III) coordinated amines are more acidic than their non-coordinated analogues and in basic conditions become good nucleophiles. This behaviour has led to the synthesis of a range of new polyamine complexes.[2,7,10,11] These polyamines, isolated from the cobalt centre, are of interest because they may bind tightly to DNA and influence replication and cell division.

The new polyamine, **20**, described here may be reduced and isolated from the complex, **5a**. Moreover, the ligand bears a pendant olefin which provides a route to further functionalisation whilst still coordinated to Co(III). For example, an interesting material might be produced by polymerising the complex on the surface of an electrode.

In order to examine the reactivity of Co(III) coordinated α-imino acids in the absence of primary amine coligands (such as ethane diamine) that react with the imino acids, the bis-bipyridine complex $[(bipy)_2Co(NHC(CH_3)COO)]^{2+}$ was synthesised. However, this complex proved to be quite unstable in base, so it is necessary to look for other complexes for reactions under these conditions. The tren complex, **36**, in which there is no possibility of forming a *mer* polyamine like **5a**, and the recently synthesised complex[17] **37** might prove useful for these purposes.




**36**




**37**

# Experimental

## *INSTRUMENTS, REAGENTS AND ANALYSES*

$^1H$ and $^{13}C$ nuclear magnetic resonance spectra of the complexes dissolved in 0.1 M DCl were acquired using a Varian Instruments Gemini 300 NMR spectrometer. Chemical shifts in $^1H$ nmr spectra are reported relative to sodium trimethylsilylpropanesulfonate (NaTPS), 0.00 ppm. Chemical shifts in $^{13}C$ nmr spectra were established relative to dioxane, 67.4 ppm Multiplicities of signals in the $^1H$ nmr spectrum are indicated by the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br). Most solvents and basic chemicals used for syntheses were analytical reagent grade. Commercial MMM $CF_3SO_3H$ was distilled before use. Dmf and dmso were dried over $CaSO_4$ before use. Ion exchange chromatography was performed with analytical grade Dowex 50Wx2 ($H^+$ form, 200 - 400 mesh) or SP Sephadex C25 ($Na^+$ form). Complexes present in the collected eluents were recovered by evaporation under water pump vacuum using a Büchi rotary evaporator (~ 20 τ), with a water bath temperature of less than 40 °C. Elemental icroanalyses were performed by the ANU Microanalytical Service. The $pK_a$ of $[(bpy)_2Co(NHC(CH_3)COO)](CF_3SO_3)_2$ was determined by Mr D. Bogsanyi.

The complexes $[(NH_3)_4Co(NHC(CH_3)COO)]Cl_2$,[1] $[(NH_3)_4Co(NC(CH_3)COO)]ClO_4$,[1] $[(NH_3)_4Co(NC(C_6H_5)COO)]ClO_4$, $[(en)_2Co(NHC(CH_3)COO)]Cl_2$[18] and $[(en)_2Co(NC(CH_3)COO)]ClO_4$[1] were prepared by previously published methods. $[(bpy)_2Co(NH_2C(CH_3)COO)](ClO_4)_2$ and $[(bpy)_2Co(NHC(CH_3)COO)](ClO_4)_2$ were first prepared by Dr P M Angus, ANU. As this work has not been published yet the synthetic procedures have been described below.

**NOTE:** ***1,4-di bromo-2-butene is a known carcinogen.***

All experiments using this chemical were performed in a fume hood and all solid and liquid residues collected in methanol and treated with concentrated ammonia solution. The resulting solution was reduced to a low volume and then disposed of using the usual method of disposal of toxic residues. All glassware and other utensils were soaked in a mixture of ammonia/methanol (20% v/v) before being washed in the usual way.

## *SYNTHESES*

### *Synthesis of $[(NH_3)_4Co(N(CH_2CH_3)C(CH_3)COO]Cl_2$ (6)*

$[(NH_3)_4Co(N{=}C(CH_3)COO]ClO_4$ (0.5 g) was dissolved in 5 $cm^3$ of dmso. Iodoethane (2 $cm^3$) was added to the solution and the resulting mixture stirred for 20 minutes at room temperature. The orange solution was poured into 120 $cm^3$ of water and extracted with dichloromethane (2 x 50 $cm^3$) to remove the excess iodoethane. The aqueous phase was then adsorbed on a Dowex column (4.5 x 14.0 cm) and washed with water to remove the dmso. An eluent of 0.1 M $Na_3PO_4$ (pH 12.3) separated the material on the column into two bands, the first red-pink and the second orange. Once the first band had reached the bottom of the column the phosphate eluent was replaced by 1 M NaCl to collect the two bands. These solutions were desalted on small columns of Dowex (2.0 x 3.0 cm); washing the adsorbed material with $H_2O$ and 0.5 M HCl before eluting the complex with a small volume of 2 M HCl. Rotary evaporation reduced the solutions to dryness. $^1H$ nmr identified the first band as the protonated starting material and the second to be the N-alkylated product; this was recrystallised from the minimum volume of warm water with the addition of a little ethanol (0.24 g, 48%). Analysis calculated for $[CoC_5H_{20}N_5O_2]Cl_2.0.5H_2O$: Co, 18.4; C, 18.7; H, 6.6; N, 21.8; Cl, 22.1. Found: C, 18.5; H, 6.7; N, 21.5; Cl, 21.9. $^1H$ nmr (0.1 M DCl): $\delta$ 3.98 (br, 3H, $NH_3$), 3.75 (ABq, 2H, $CH_2$), 3.56 (br, 6H, $2xNH_3$), 3.27 (br, 3H, 4 x $NH_3$), 2.48 (s, 3H, $=C-CH_3$), 1.31 (t, 3H, $CH_2-CH_3$). $^{13}C$ nmr (0.1 M DCl): $\delta$ 181.5 (COO), 174.5 (C=N), 49.1 ($CH_2$), 22.6 ($=C-CH_3$), 18.7 ($CH_2-CH_3$).

### *Reaction of $[N_4Co(NC(CH_3)COO]^+$ ($N_4$ = $(NH_3)_4$, $(en)_2$) with a variety of haloalkanes*

The synthetic procedure described above was repeated, by reacting a variety of haloalkanes with $[(en)_2Co(N{=}C(CH_3)COO]ClO_4$ and/or $[(NH_3)_4Co(N{=}C(CH_3)COO]ClO_4$ and/or $[(NH_3)_4Co(N{=}C(C_6H_5)COO]ClO_4$. The reactions involving diiodomethane, 1,2-dibromo- and 1,2-diiodo- ethane and 1,3-

diiodopropane are described in Chapter 2. $[(en)_2Co(N{=}C(CH_3)COO]ClO_4$ and 1,3-diiodopropane were also mixed in the presence of DMAP (4-dimethylaminopyridine) without alkylation of the imine-N; only the starting material was retrieved from the reaction mixture. Other reactions with haloalkanes including 2-iodoethanol and 1-chloro, 3-bromo-propane did not succeed in alkylating the imine-N. The results of these reactions are summarised in Table 1.

***Synthesis of $[(NH_3)_4Co(N(CH_2COOH)C(CH_3)COO)]Cl_2$ (7)***

Iodoacetic acid was converted to the corresponding sodium salt by dissolving a portion (2.67 g) in a minimum volume of water (3 to 4 $cm^3$) and then adding sodium bicarbonate (1.33 g). The resulting solution was reduced to dryness by rotary evaporation and the white solid, sodium iodoacetate, dried under vacuum in the presence of $P_2O_5$.

The deprotonated complex $[(NH_3)_4Co(N{=}C(CH_3)COO)]ClO_4$ (1.50 g) was added to the sodium iodoacetate obtained by the above method and the mixture dissolved in dry dmso (20 $cm^3$). The resulting deep red-brown coloured solution was stirred at room temperature for 20 minutes before being diluted with 300 $cm^3$ of water and adsorbed on a Sephadex column (5.5 x 22.0 cm). Elution with 2 M NaCl provided two bands, both orange:

*Band 1*: was desalted by adsorbing the complexes on a Dowex column (5.0 x 2.5 cm), washing it with $H_2O$ (200 $cm^3$) and 0.5 M HCl (200 $cm^3$), and elution with 1 M HCl. Rotary evaporation led to an orange solid which was recrystallised by dissolving it in a minimum volume of water, adding ethanol until the solution appeared slightly cloudy and then refrigerating the solution to complete crystallisation. The orange crystals of the product were collected by vacuum filtration, washed with ethanol and ether and dried under vacuum (1.09 g, 66%). Analysis calculated for $[CoC_5H_{18}N_5O_2]Cl_2 \cdot 0.5H_2O$: Co, 16.8; C, 17.1; H, 5.5; N, 20.0; Cl, 20.2. Found: Co, 16.5; C, 17.1; H, 5.6; N, 19.8; Cl, 20.6. $^{1}$H nmr (0.1 M DCl): δ 3.98 (br, 3H, $NH_3$), 3.72 (s, 2H, $CH_2$), 3.70 (br, 6H, 2 x $NH_3$), 3.36 (br, 3H, $NH_3$), 2.43(s, 3H, $CH_3$). $^{13}$C nmr (0.1 M DCl): δ 182.1 (Co-COO), 171.1 ($CH_2$-COO), 167.9 (C=N), 52.4 ($CH_2$), 15.8 ($CH_3$).

*Band 2:* was desalted in the manner described above. $^{1}$H nmr of the resulting solid residue identified it to be the protonated starting material.

***Reaction of [(en$_2$)Co(N=C(CH$_3$)COO)] ClO$_4$with trans-1,4-dibromo-2-butene***

A mixture consisting of [(en)$_2$Co(NC(CH$_3$)COO)]ClO$_4$ (1.50 g) and *trans*-1, 4-dibromo-2-butene (4.50 g) in dmf (75 cm$^3$) was stirred at 40$^{o}$C for 2 hours. The resulting dark orange-brown solution was diluted with water (200 cm$^3$) and the excess haloalkane removed by washing with chloroform (3 x 50 cm$^3$). The orange aqueous phase was adsorbed to a Dowex column (3 x 22 cm) and washed with water (~200 cm$^3$) and then 0.5M HCl (200 cm$^3$). Elution of the complexes with 2M HCl generated three orange bands. The eluent in each of the bands was collected, the solvent removed by rotary evaporation and the complexes identified by nmr as:

*Band 1:* the protonated starting material (0.68 g)

*Band 2:* the adduct **5a**, which was recrystallised from water by adding ethanol and refrigerating the resulting mixture for 4 days. The product was collected by vacuum filtration, washed with a little ethanol and dried under vacuum (0.44g, 28%). Analysis calculated for [CoC$_{11}$H$_{24}$N$_5$O$_2$]Cl$_2$.H$_2$O: Co, 14.8; C, 32.5; H, 6.5; N, 17.2; Cl, 17.5. Found: Co, 14.6; C, 32.8; H, 6.4; N, 17.2; Cl, 17.9. $^{1}$H nmr (0.1 M DCl): δ 6.3 - 4.7 (br, 7H, en-NH$_2$), 6.15 (m, 1H, CH-CH$_{\mathbf{X}}$=CH$_2$), 5.64 (d, 1H, CH=CH$_{\mathbf{A}}$H), 5.61 (d, 1H, CH=CH$_{\mathbf{B}}$H), 4.50 (m, 1H, =CH-CH), 4.37 (m, 2H, =N-$CH_2$), 3.2 -2.6 (br, 8H, en-$CH_2$), 2.57 (s, 3H, $CH_3$). $^{13}C$ nmr (0.1 M DCl): $\delta$ 181.5 (COO), 173.8 (C=N), 129.6 (C=), 125.2 (C=), 65.7 (N-CH-CH=), 55.6 (=N-$CH_2$-CH), 49.3 (en-$CH_2$), 46.3 (en-$CH_2$), 44.2 (en-$CH_2$), 19.8 ($CH_3$).

*Band 3:* the binuclear species formed by the self condensation of coordinated alanine imine. Characterisation of this species was described in the previous chapter.

***Synthesis of crystals of the bromide salt of 5a for X-ray crystallography***

A small quantity of **5a** (~ 0.05 g) was dissolved in a small volume of water (about 10 $cm^3$). Potassium bromide (excess) was added to this solution before it was left to evaporate slowly at about 25 $^oC$. After some time (about three weeks) small, regular, orange crystals formed. These were collected by vacuum filtration, air dried and submitted for X-ray crystallographic analysis.

***Synthesis of [(bpy)$_2$Co(NH$_2$C(CH$_3$)COO)](ClO$_4$)$_2$ (29)***

$CoCl_2.6H_2O$ (2.4 g) and alanine (1.3 g) were dissolved in a minimum volume of $H_2O$. A solution of bipyridine (2.4 g) in methanol (10 $cm^3$) was added to this mixture. When all solid material had dissolved, $PbO_2$ (4.0 g) was added and the mixture heated on a steam bath for 1 hour. After this time the mixture was diluted to 100 $cm^3$ with $H_2O$ and filtered before passing it through a Dowex column (2.5 x 10.0 cm). After washing the adsorbed complexes with $H_2O$ (200 $cm^3$) and 0.5 M HCl (200 $cm^3$) the complexes were eluted using 2 M HCl. Three major bands separated:

*Band 1:* was orange in colour and the major fraction of the complexes. The acid was removed by rotary evaporation and nmr spectroscopy identified it as the product, $[(bpy)_2Co(NHC(CH_3)COO)]^{2+}$. It was recrystallised from 1 M $HClO_4$ with the addition of a little $NaClO_4$ (3.34 g, 66%). Analysis calculated for $[CoC_{23}H_{22}N_5O_2](ClO_4)_2.0.5H_2O$: Co, 9.0; C, 42.0; H, 3.4; N, 10.6; Cl, 10.8. Found: Co, 8.8; C, 41.9; H, 3.7; N, 10.4; Cl, 11.0. Signals from both diastereoisomers were present in the nmr spectra. $^1H$ nmr (0.1 M DCl): $\delta$ 8.6 - 7.8 (m, H, bipy-CH); ;3.93, 3.61, (quintet, 2H, 2 x $NH_2$-CH-$CH_3$); 1.34, 1.57 (d, 6H, 2 x $CH_3$). $^{13}C$ nmr (0.1 M DCl): $\delta$ 188.9, 188.2 (COO); 157.6, 151.9, 151.5, 143.2, 129.1, 125.1 (bipy-CH); 54.5, 54.3 ($NH_2$-CH-$CH_3$); 19.0, 18.9 ($CH_3$).

*Band 2:* was yellow-brown in colour and the smallest of the three bands. It was removed from the column using 6 M HCl. After reducing the eluent to dryness by rotary evaporation the residual solid was identified by nmr spectroscopy as $[(bpy)_3Co]Cl_3$.

*Band 3:* contained a quantity of brown coloured material which remained adsorbed to the top of the column.

***Synthesis of $[(bpy)_2Co(NHC(CH_3)COO)](ClO_4)_2$ (30)***

A quantity of $[(bpy)_2Co(NH_2C(CH_3)COO)](ClO_4)_2$ (0.50 g) was dissolved in dmf (5 $cm^3$) and the resulting solution chilled in ice. Thionyl chloride (5 $cm^3$) was added dropwise to the cold, stirred solution. After the addition was complete the mixture was stirred for 10 minutes and then removed from ice and stirred for a further 30 minutes as the solution warmed to ~ 25 $^oC$. It was then poured into an ice/water slurry (~ 300 $cm^3$). Once the ice had melted the orange solution was filtered and then passed through a Dowex column (2.5 x 15 cm). The adsorbed material was washed with water (~200 $cm^3$) and 1 M HCl (~100 $cm^3$) before being eluted with 2 M HCl. A single orange band was collected and recrystallised from 1 M $HClO_4$ with the addition of a little $NaClO_4$ and a small volume of ethanol (0.45 g, 90%). Analysis calculated for $[CoC_{23}H_{22}N_5O_2](ClO_4)_2.0.5H_2O$: Co, 8.9; C, 41.5; H, 3.2; N, 10.5; Cl, 10.7. Found: Co, 8.8; C, 41.3; H, 3.4; N, 10.3; Cl, 10.1. $^1H$ nmr (0.1 M DCl): $\delta$ 8.7 -

7.8 (m, H, bipy-CH), 2.54 (s, 3H, $CH_3$). $^{13}C$ nmr (0.1 M DCl): δ 185.7 (COO); 174.2 (C=N); 157.6, 151.6, 142.9, 129.5, 124.5 (bipy-CH), 22.2 ($CH_3$).

### *Synthesis of $[(bpy)_2Co(NHC(CH_3)COO)](CF_3SO_3)_2$*

The eluent containing $[(bpy)_2Co(NHC(CH_3)COO)]Cl_2$ from the preceding synthesis was reduced to dryness by rotary evaporation. The residue was dried overnight under vacuum in the presence of $P_2O_5$. The resulting solid was dissolved in anhydrous triflic acid (5 $cm^3$) and stirred at 25 °C for 3 hours. A steady stream of dry nitrogen bubbling through the solution drove off the HCl that formed. The triflate salt was precipitated by pouring the solution into vigorously stirring ether (300 $cm^3$). The ether was decanted and the sticky orange solid dissolved in a minimum volume of acetone and reprecipitated by pouring the solution into ether (400 $cm^3$). The orange powder was collected by vacuum filtration, washed with ether and dried under vacuum over silica (0.50 g, 97%). Analysis calculated for $[CoC_{23}H_{22}N_5O_2](CF_3SO_3)_2.2H_2O$: Co, 7.7; C, 37.8; H, 3.3; N, 8.9; F, 14.4; S, 8.1. Found: Co, 7.6; C, 37.8; H, 3.4; N, 8.8; F, 14.1; S, 7.9. $^1H$ nmr, $^{13}C$ nmr (0.1 M DCl): As described above.

### *Determination of the $pK_a$ of $[(bpy)_2Co(NHC(CH_3)COO)](CF_3SO_3)_2$*

Replicates of 5.55 x $10^{-5}$ and 3.83 x $10^{-5}$ moles of $[(bpy)_2Co(NHC(CH_3)COO)](CF_3SO_3)_2.0.5H_2O$ were dissolved in 10.0 $cm^3$ of $H_2O$. The solution was titrated with 0.10 M NaOH in 0.02 $cm^3$ aliquots at 25 °C, using a Radiometer 108 pH meter. The $pK_a$ was calculated on the merged data using the computer program Superquad,[18] resulting in a value of 9.58 (SD = 0.015). Data related to this experiment are included in the appendices.

### *Synthesis of $[(bpy)_2Co(N{=}C(CH_3)COO)]^+$ (31)*

A sample of $[(bpy)_2Co(NHC(CH_3)COO)]Cl_2$ (0.12 g) was dissolved in 1 M NaOH (0.25 $cm^3$) to form a deep brown solution. After 20 seconds $NaClO_4$ (1.0 g) was added. After stirring vigorously for about one minute the remaining $NaClO_4$ was removed by filtration. Addition of isopropanol and a little ether to the filtrate resulted in a peach coloured precipitate which was collected by vacuum filtration (0.05 g). This was dried in the presence of $P_2O_5$ and under vacuum for about 4 hours before it was used in the preparation of the N-methyl analogue (described below).

***Synthesis of [(bpy)$_2$Co(N(CH$_3$)C(CH$_3$)COO)]Cl$_2$ (32)***

A sample of [(bpy)$_2$Co(N=C(CH$_3$)COO)]ClO$_4$ (0.05 g), isolated using the procedure described above, was dissolved in dmso (2 cm$^3$). Methyl iodide (2 cm$^3$) was added to this solution. Once the solution's colour had changed from brown to orange (~ 15 minutes) it was diluted with water (50 cm$^3$) and washed with dichloromethane (2 x 20 cm$^3$) to get rid of the excess iodomethane. The aqueous phase was passed down a Dowex column (2.5 x 15.0 cm), the adsorbed complexes washed with water (100 cm$^3$) and 0.5 M HCl (50 cm$^3$) and then eluted with 2 M HCl. The second of the two bands proved to contain the N-methyl complex, **32**. The solvent was removed by rotary evaporation to give an orange solid (~0.02 g, 43%). It was characterised only by nmr spectroscopy. $^1$H nmr (0.1 M DCl): $\delta$ 8.8 - 7.8 (m, bipy-CH), 2.78 (s, 3H, =N-CH$_3$), 2.47 (s, 3H, =C-CH$_3$). $^{13}$C nmr (0.1 M DCl): $\delta$ 184.9 (COO); 174.2 (C=N); 157.2, 151.8, 143.2, 130.0, 124.9 (bipy-CH), 40.6 (N-CH$_3$), 22.2 (=C-CH$_3$), .

***Reaction of [(bpy)$_2$Co(NHC(CH$_3$)COO)](CF$_3$SO$_3$)$_2$ with trans-1,4-dibromo-2-butene***

A sample of [(bpy)$_2$Co(NHC(CH$_3$)COO)](CF$_3$SO$_3$)$_2$.2H$_2$O, (0.50 g) was dissolved in dry dmf (5 cm$^3$). Diisopropylethylamine (0.09 g), also dissolved in dry dmf (1 cm$^3$), was added to this solution, followed by *trans*-1,4-dibromo-2-butene (0.42 g). The reaction mixture, almost black in colour, was stirred at 25 $^o$C for 4 hours. During this time samples of the mixture were passed down small columns of C25 Sephadex (1 x 4 cm) to monitor the reaction's progress. Three orange bands formed (0.05 M sodium citrate used as eluent). The reaction was quenched by diluting the solution with water (200 cm$^3$) and adding 2 M acetic acid until a pH of 5 was reached. Excess *trans*-

1,4-dibromo-2-butene was removed by washing the aqueous phase with dichloromethane (2 x 50 $cm^3$). The aqueous phase was passed down a Dowex column (2.5 x 6.0 cm). After washing the adsorbed material with water (100 $cm^3$), 2 M HCl was used to remove a faint orange band (~5% of total material). Removal of the solvent by rotary evaporation left a dark green solid which was discarded. The remainder of the material was eluted from the column with 4 M HCl and evaporated to dryness. The residue was dissolved in water (30 $cm^3$) and adsorbed to the top of a C25 Sephadex column (2.5 x 17.0 cm). Elution of the adsorbed complexes with 0.05 M sodium citrate yielded a number of bands:

*Band 1:* a minor species; yellowish colour but turned green after desalting on a small Dowex column and reducing the acid eluent to dryness by rotary evaporation. Discarded. (~15%).

*Band 2:* the major species and orange in colour. After desalting it on Dowex and reducing it to dryness by rotary evaporation, nmr spectroscopy identified it as the (protonated) starting material. (~55%).

*Band 3:* a minor, orange band. Nmr spectra of the desalted complex implied a mixture of species, most probably analogues of the binuclear complexes described in Chapter 2. (~10%).

*Band 4:* yellow, in colour, it remained strongly adsorbed to the top of the Sephadex column. (~15%).

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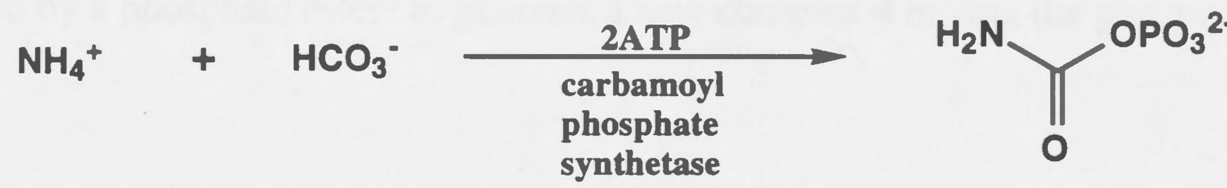
# CHAPTER 6

## Synthesis and Hydrolysis of Phosphate Derivatives using Co(III) Templates

## Introduction

The previous chapters have discussed the way in which Co(III) complexes may be used as tools for the synthesis or modification of polyamines and of amino acids. The material presented in this chapter will deal with the way in which Co(III) complexes may be used to synthesise or modify biologically significant phosphates and phosphate esters. In particular, it will examine complexes capable of synthesising pyrophosphate, $P_2O_7^{4-}$ and hydrolysing carbamoyl phosphate, **1**, and DNA. As such, these complexes and their reactivity have implications for understanding the behaviour of metalloenzymes which perform these functions in biological systems.

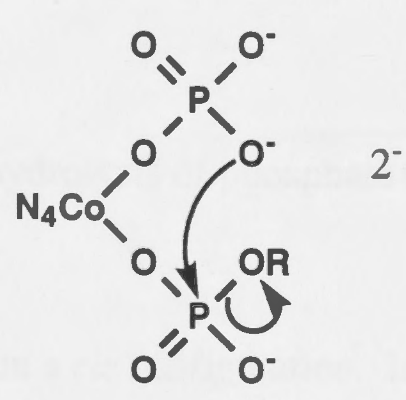



**1**

Figure 1: Biosynthesis of Carbamoyl Phosphate

### *Synthesis of pyrophosphate by Co(III) complexes*

To date, the vast majority of research dealing with metal promoted reactions of phosphate derivatives has focused on the hydrolysis of a phosphate group or an ester group from the remainder of the molecule.[1] Very few reports have dealt with the reverse and equally




**2**

important reactions: the synthesis of pyrophosphate and other polyphosphates and polyphosphate esters. Substitutionally inert tetraamine Co(III) complexes could be expected to provide a template for pyrophosphate synthesis to take place, as depicted in **2**. The metal ion should also activate the phosphorus centre of a coordinated phosphate ester towards attack by an intramolecular nucleophile, such as oxygen from the adjacent phosphate ligand.[2] The results of a preliminary investigation into the use of such Co(III) complexes in synthetic and mechanistic studies of the formation of pyrophosphate are described in the following pages.

***Hydrolysis of phosphate esters by Co(III) complexes***

Cobalt(III) hydroxo aqua complexes, **3**, have been used extensively in the hydrolysis of phosphate esters.[1,2,3] The water molecule can be made quite labile so that it is readily displaced by a phosphate ester[3] to generate a new complex **4** having the phosphate ester and

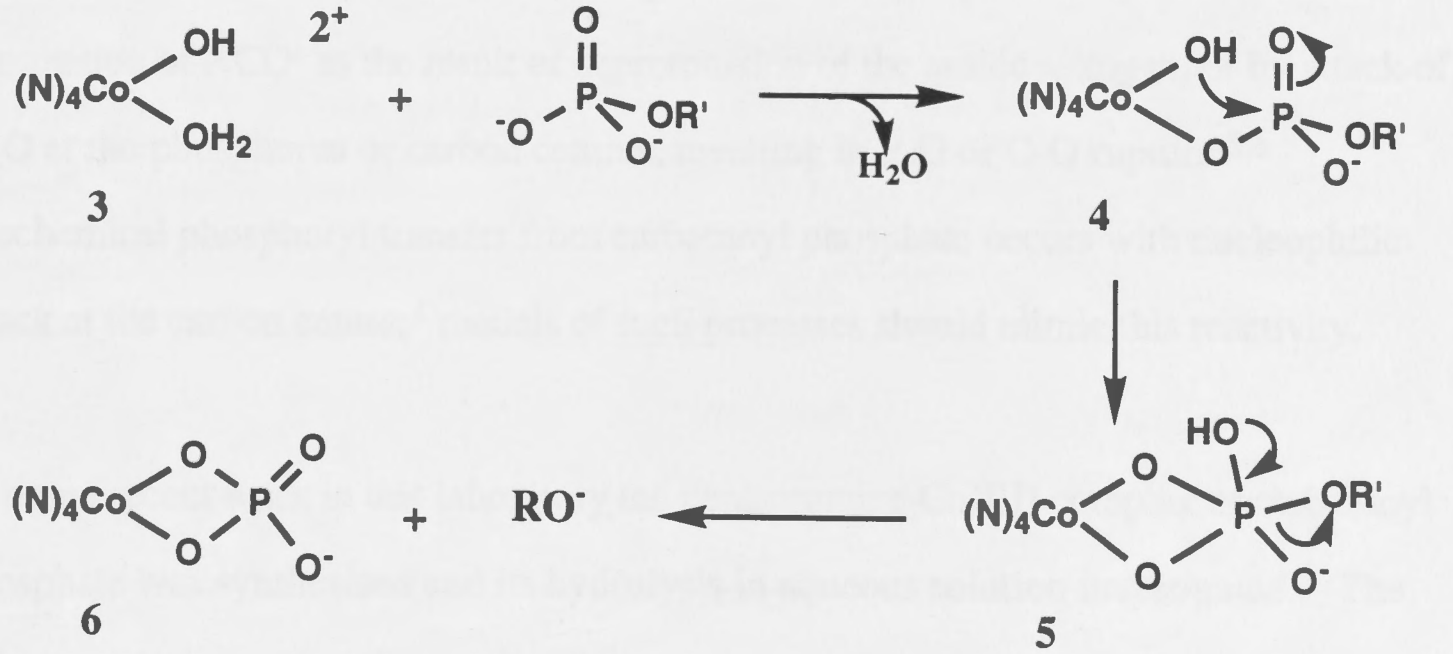



Figure 2: Intramolecular hydrolysis of phosphate esters coordinated to Co(III).

coordinated hydroxide arranged in a *cis* configuration. Intramolecular attack on the phosphorus atom by the bound $OH^-$ leads to rupture of a P-O bond and liberation of an alcohol from the complex, **6**. The hydrolysis of the ester occurs relatively rapidly at near neutral conditions, in spite of the formation of a strained 4-membered chelate ring and reduction of basicity of $OH^-$ on coordination to Co(III),[2] so the intramolecular pathway must be very efficient. Several complexes of the type $[N_4Co(OH_2)(OH)]^{2+}$ have now been used to study aspects of the hydrolysis of two bio-molecules: carbamoyl phosphate and plasmid (Φpuc9) DNA.

*Hydrolysis of carbamoyl phosphate*

Carbamoyl phosphate is an important intermediate in the biosynthesis and degradation of nitrogenous substances such as amino acids and nucleotides.[4] For example, it is a donor of the carbamoyl moiety to aspartate in the initial step of the synthesis of UMP (uridine monophosphate). Its ubiquitous nature has made it an attractive subject for study[5] and the development of efficient methods for such transfers are of importance for chemistry in general.

Hydrolysis of carbamoyl phosphate in aqueous solution may occur via unimolecular elimination of $NCO^-$ as the result of deprotonation of the amide nitrogen, or by attack of $H_2O$ at the phosphorus or carbon centres, resulting in P-O or C-O rupture.[5,6] Biochemical phosphoryl transfer from carbamoyl phosphate occurs with nucleophilic attack at the carbon centre;[4] models of such processes should mimic this reactivity.

In some recent work in this laboratory the pentaammine Co(III) complex of carbamoyl phosphate was synthesised and its hydrolysis in aqueous solution investigated.[6] The only non-phosphate species produced was cyanate. The elimination followed as the result of deprotonation of the amide nitrogen during the reaction, Figure 3.

Hydrolysis of **7** was also performed in the presence of $[trpnCo(OH_2)(OH)]^{2+}$ to examine the effect of extra metal complex cations on the reaction. The rate of reaction increased, from $5.5 \times 10^{-5}\ s^{-1}$ in the absence of the trpn complex, to approximately $1 \times 10^{-2}\ s^{-1}$ in the presence of the complex.[6] However, it was not possible to unequivocally identify the carbon products of the reaction and hence the mechanism by which the hydrolysis occurred. This work is continued here with a study of the reaction (particularly the products) between $[tamenCo(OH_2)(OH)]^{2+}$ and $[(NH_3)_5Co(OPO_3CONH_2)]^+$.

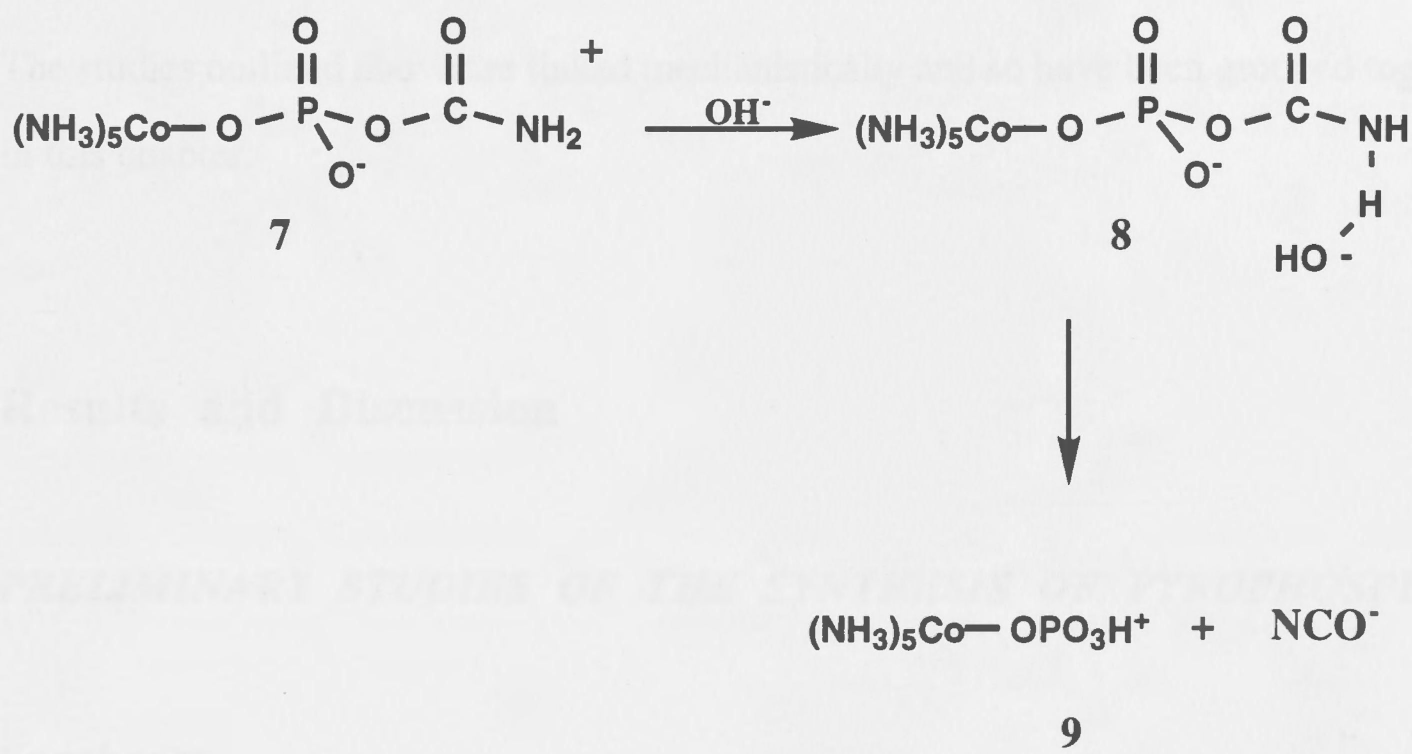


Figure 3: Hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$ by elimination.

*Hydrolysis of Plasmid DNA*

The development of reagents capable of cleaving the polymeric phosphodiester nucleic acids is very popular because of their potential applications as models of nucleases, new therapeutic agents and tools for molecular biology.[7] At present, most of these reagents cleave the nucleic acids by generation of radicals[8] or by oxidative cleavage followed by reduction;[7b] relatively few hydrolyse the phosphodiester chains in the manner of enzymes such as restriction endonucleases.[9] These include hydrolysis of RNA,[10] and hydrolysis of deoxy-dinucleotides and single stranded DNA using labile lanthanide-based systems.[11]

Previous work has demonstrated the efficacy of Co(III) hydroxo aqua complexes for the hydrolysis of phosphate diesters.[1c,12,23] The series of experiments described here investigated the ability of a number of these complexes to hydrolyse DNA.

The studies outlined above are linked mechanistically and so have been grouped together in this chapter.

## Results and Discussion

### *PRELIMINARY STUDIES OF THE SYNTHESIS OF PYROPHOSPHATE*

#### *Syntheses*

*Synthesis of* $[(tn)_2Co(OPO_3H)(OPO_2HOC_6H_4NO_2)]$

Disodium p-nitrophenylphosphate was converted to the corresponding acid ($H_2PNPP$) by ion exchange chromatography. The solvent was removed and a $^{31}P$ nmr spectrum obtained to ensure that the molecule had not hydrolysed or self condensed ~~in the meantime~~. $H_2PNPP$ and an equimolar quantity of $[tn_2Co(O_2PO_2)]$ were dissolved in a minimum volume of water and left at 25 °C for five minutes. Under the acidic conditions, the chelate ring of $[(tn)_2Co(O_2PO_2)]$ opened and $PNPP^{2-}$ coordinated to the metal centre, **11**. The reaction was quenched by precipitating the product with ethanol. Characterisation of this material by $^{31}P$ nmr spectrometry, uv-vis spectroscopy (at 398 nm) and elemental microanalysis indicated the presence of small quantities of impurities, including some p-nitrophenylate ($PNP^-$), implying some hydrolysis of $PNPP^{2-}$ had occurred. The level of these impurities were reduced by dissolving the complex in a minimum quantity of water and reprecipitating it with ethanol. This complex displayed $^{31}P$ chemical shifts at 4.8 and 11.2 ppm which are characteristic of $PNPP^{2-}$ and $PO_4^{3-}$ bound monodentate to the Co(III) amine complex.

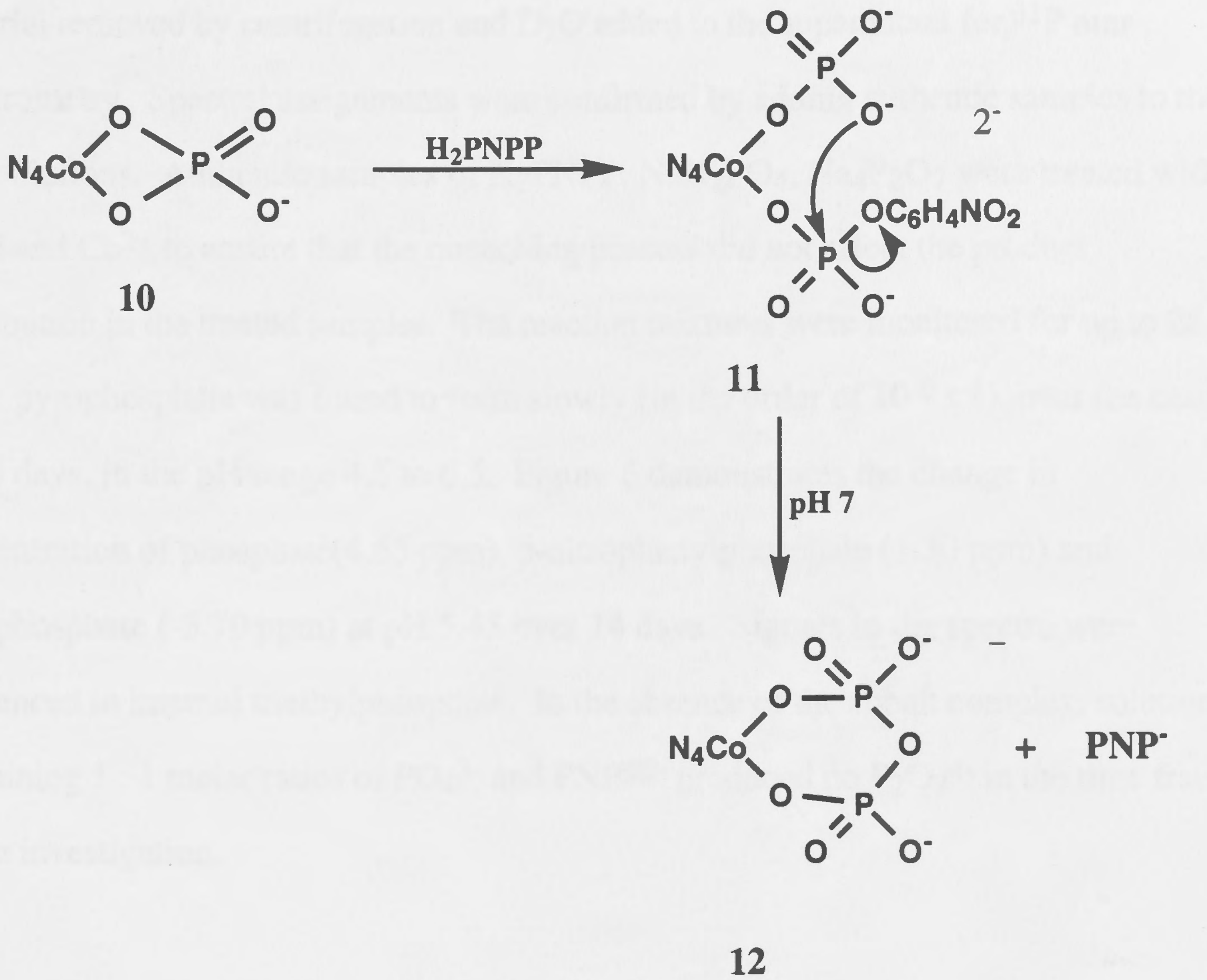


Figure 4: Synthesis of pyrophosphate on a Co(III) template.

*Synthesis and Identification of $[(tn)_2Co(P_2O_7)]^-$*

The synthesis of pyrophosphate by **11** is described in Figure 4. Nucleophillic oxygen, from coordinated phosphate attacks the phosphorus atom of *cis*-coordinated $PNPP^{2-}$ with a resulting loss of $PNP^-$. The product, **12**, contains chelated pyrophosphate in a stable, six-membered ring.[12] $^{31}P$ nmr spectrometry was used to monitor the progress of the reaction over the pH range 2.0 to 7.50, Table 1. The pH of the unbuffered solution remained at pH ~ 4.5 during the period in which the reaction was monitored and the product distribution from this reaction has also been included in Table 1.

Signals in the $^{31}P$ nmr spectra of the reaction mixture were quite broad and some overlap occurred (especially over extended periods of time, as small quantities of $Co^{2+}$ formed) making it difficult to obtain accurate integrals. Consequently, samples of the reaction mixture (0.05 M in complex) were quenched[13] by the addition of KCN and $Co^{2+}$, solid material removed by centrifugation and $D_2O$ added to the supernatant for $^{31}P$ nmr spectrometry. Spectral assignments were confirmed by adding authentic samples to the nmr solutions. Authentic samples of $H_2PNPP$, $NaH_2PO_4$, $Na_4P_2O_7$ were treated with KCN and $Co^{2+}$ to ensure that the quenching process did not affect the product distribution in the treated samples. The reaction mixtures were monitored for up to 28 days; pyrophosphate was found to form slowly (in the order of $10^{-6}$ $s^{-1}$), over the course of 14 days, in the pH range 4.5 to 6.5. Figure 5 demonstrates the change in concentration of phosphate(4.65 ppm), p-nitrophenylphosphate (1.30 ppm) and pyrophosphate (-3.70 ppm) at pH 5.45 over 14 days. Signals in the spectra were referenced to internal triethylphosphate. In the absence of the cobalt complex, solutions containing 1 : 1 molar ratios of $PO_4^{3-}$ and $PNPP^{2-}$ produced no $P_2O_7^{4-}$ in the time frame of the investigation.

At the beginning of the reaction the proportion of $PO_4^{3-}$ to $PNPP^{2-}$ should have been 50 : 50. However, there was always an excess of $PO_4^{3-}$, due to some independent hydrolysis of $PNPP^{2-}$. Pyrophosphate was synthesised in the pH range 4.5 - 6.22, most probably because of protonation and partial neutralisation of the phosphate ligands, leading to a corresponding loss of $PNPP^{2-}$ in the quenched samples. Generation of pyrophosphate was coupled with a decrease in the amount of $PNPP^{2-}$ in reaction solutions. However, the loss of $PNPP^{2-}$ was greater than the synthesis of $P_2O_7^{4-}$, and occurred without any detectable formation of $P_2O_7^{4-}$ at pH 2.0 and 7.50. This is most likely to be a result of competing reactions such as intermolecular hydrolysis of coordinated $PNPP^{2-}$ during the experiment, Figure 6.[2]

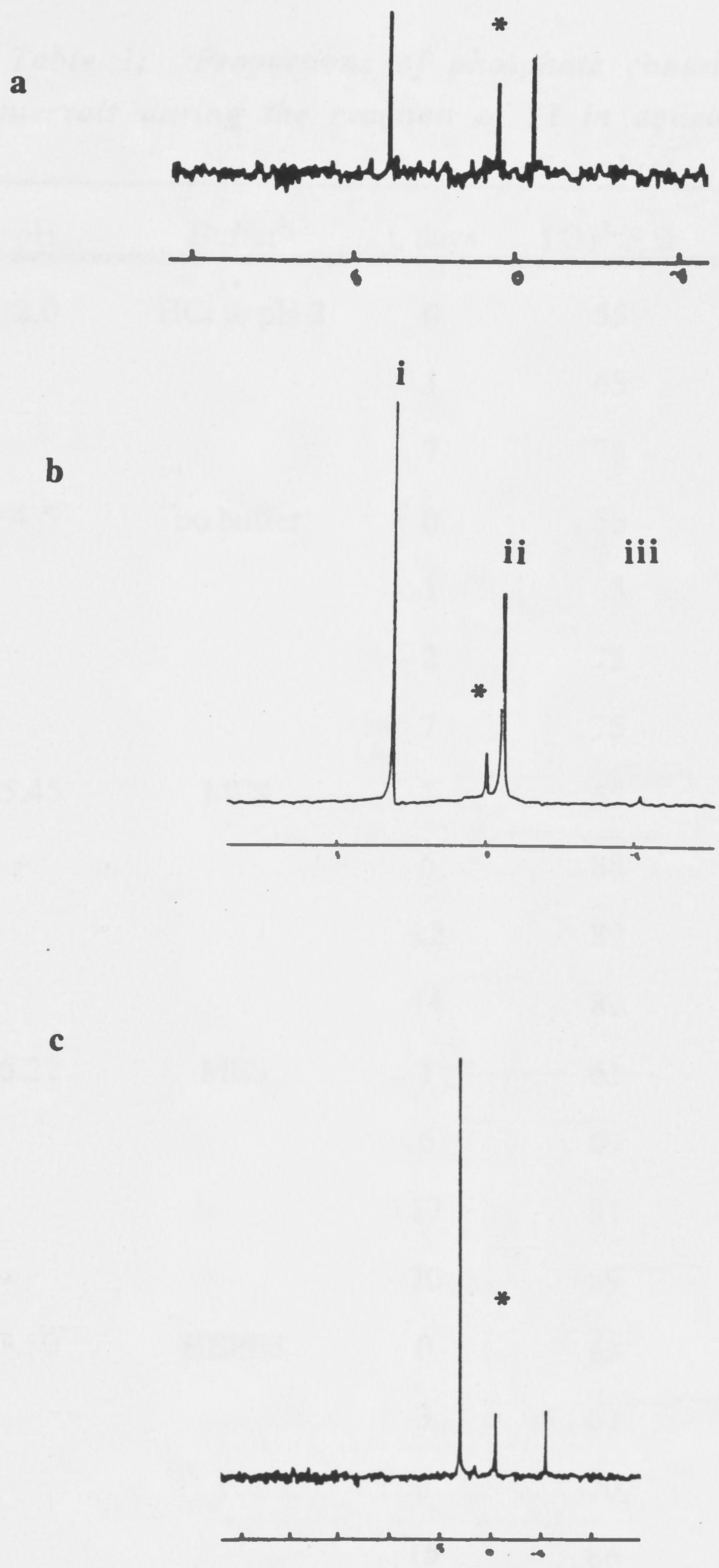



Figure 5: Formation of chelated pyrophosphate by **11** at pH 5.45. Each sample was quenched with $CN^-/Co^{2+}$ before the $^{31}P$ nmr spectrum ($D_2O/H_2O$, *TEP as internal standard) was acquired. **i**: Pi, **ii**: $PNPP^{2-}$, **iii**: PPi.

***Table 1: Proportions of phosphate containing species in obtained at intervals during the reaction of 11 in aqueous solutions over a range of pH values***

| pH | Buffer[b] | t, days | $PO_4^{3-}$,[c] % | $PNPP^{2-}$,[c] % | $P_2O_7^{4-}$,[c] % |
|---|---|---|---|---|---|
| 2.0 | HCl to pH 2 | 0 | 55 | 45 | 0 |
| | | 1 | 65 | 35 | 0 |
| | | 7 | 78 | 22 | 0 |
| 4.5 | no buffer | 0 | 56 | 44 | 0 |
| | | 1 | 63 | 37 | 0 |
| | | 2 | 75 | 21 | 5 |
| | | 7 | 75 | 16 | 9 |
| 5.45 | MES | 1 | 65 | 35 | 0 |
| | | 6 | 88 | 9 | 3 |
| | | 12 | 89 | 2 | 10 |
| | | 14 | 87 | 0 | 13 |
| 6.22 | MES | 1 | 61 | 39 | 0 |
| | | 6 | 69 | 27 | 4 |
| | | 17 | 91 | 4 | 5 |
| | | 20 | 89 | 5 | 6 |
| 7.50 | HEPES | 0 | 63 | 37 | 0 |
| | | 3 | 67 | 32 | 0 |
| | | 8 | 70 | 30 | 0 |
| | | 15 | 86 | 14 | 0 |

[a]Temperature of reaction: 25 °C. [b]Buffer concentration is 0.5 M. [c]Percentage of each species determined by comparison of the integrals of each of the peaks due to $PO_4^{3-}$, $PNPP^{2-}$ and $P_2O_7^{4-}$. Estimated error in percentage proportion of phosphate ligands: 15%.

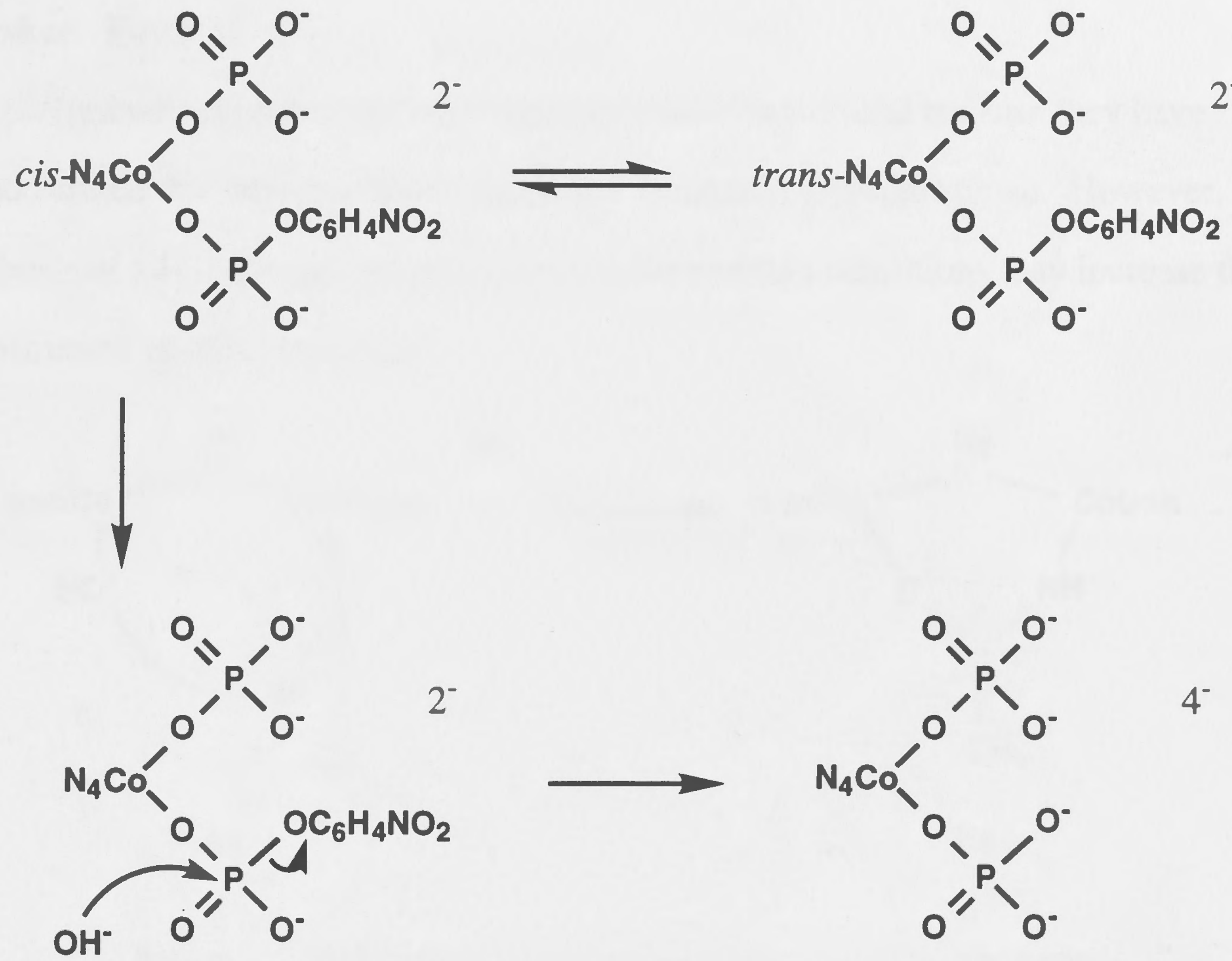
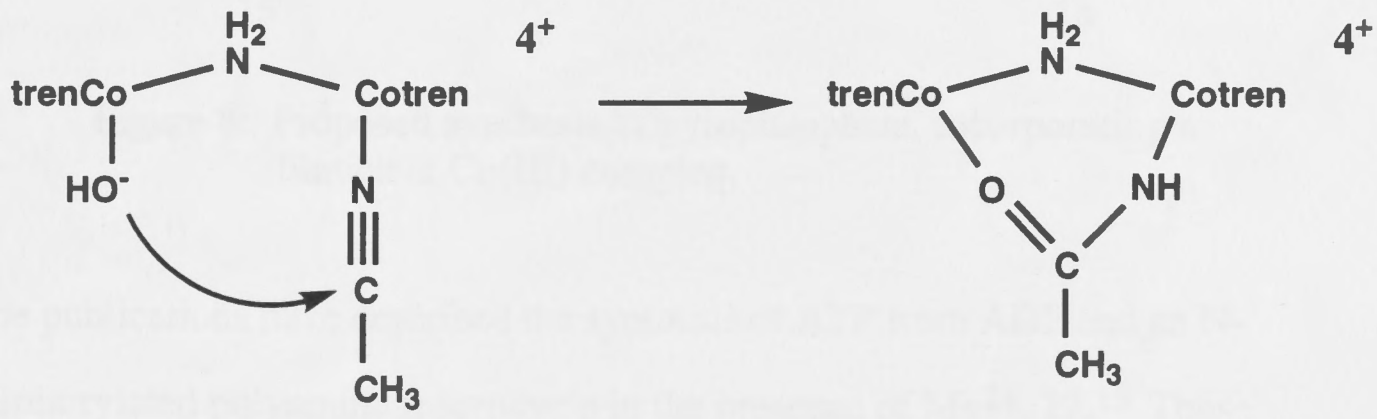


Figure 6: Reactions which compete with the synthesis of pyrophosphate.

Other reactions besides hydrolysis of $PNPP^{2-}$ will lower the yield of pyrophosphate from a system such as **11**. For example, the rate of formation of pyrophosphate proves so slow that equilibria such as the dissociation of $PO_4^{3-}$ and $PNPP^{2-}$ become significant. It is likely that in the $tn_2Co(III)$ system that both entities form and dissociate faster than pyrophosphate synthesis. Without the complex holding the two substrates in close proximity and partially neutralising their charge, pyrophosphate synthesis will be very difficult. Moreover, dissociation of the phosphate ligands can lead to the original complex $[(tn)_2Co(H_2O)(OH)]^{2+}$ which has been used to hydrolyse Co(III)-coordinated pyrophosphate in models of yeast inorganic pyrophosphatase.[12] A further complication arises when the complex equilibrates between the *cis* and *trans* isomers. Intramolecular pyrophosphate synthesis would not be possible in the *trans* isomer.

***Further Work***

The preliminary experiments described above have been useful because they have demonstrated that complexes of Co(III) can synthesise pyrophosphate. However, the synthesis of **12** is slow and some changes to the reaction conditions may increase the rate of formation of pyrophosphate.

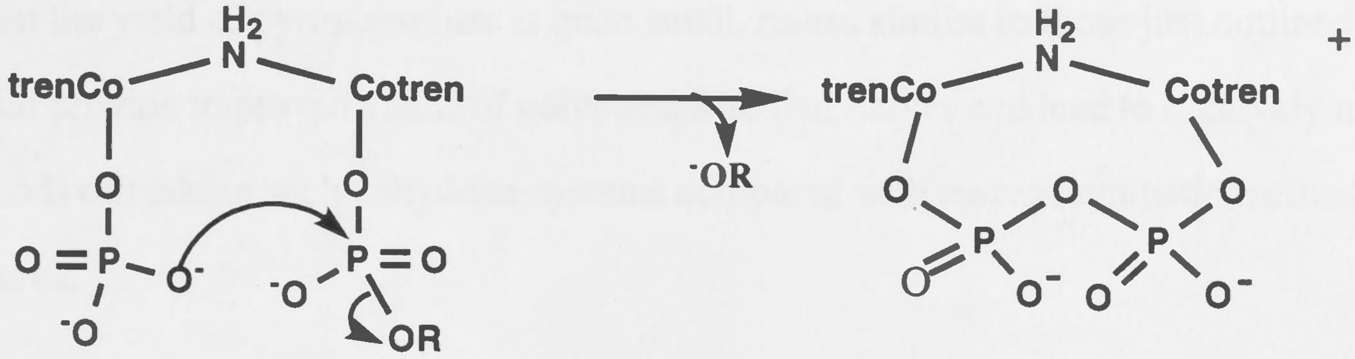


13 14

Figure 7: Intramolecular hydrolysis of acetonitrile, promoted by a binuclear Co(III) complex.

For example, p-nitrophenylphosphate might be replaced by esters such as 1,4-dinitrophenylphosphate or diesters such as bis(1,4-dinitrophenylphosphate) or ethyl-4-nitrophenylphosphate which incorporate better leaving groups and which would consequently increase the rate of reaction. Another prospective route involves derivatives of binuclear, μ-amido linked, Co(III) complexes such as those which have previously been used in the hydrolysis of coordinated acetonitrile, **13**.[14] At physiological pH the charge on the phosphate groups could be expected to be neutralised by the Co(III) centres and Dreiding models have demonstrated that the positioning of the phosphate groups facilitates attack of oxygen from $PO_4^{3-}$ on the phosphorus of $PNPP^{2-}$, Figure 8.

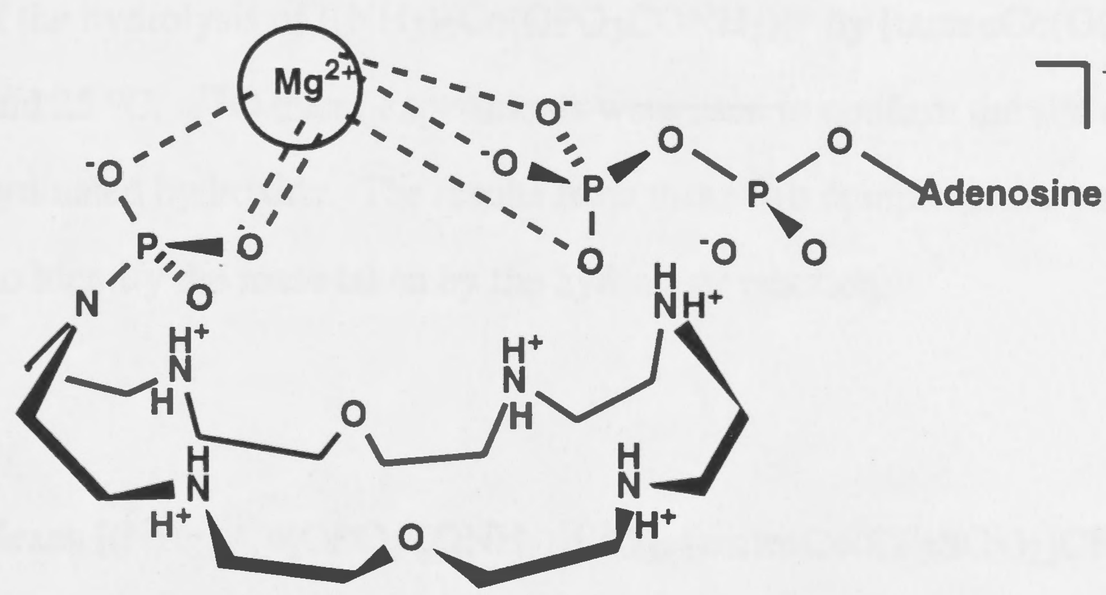
In fact, if the binuclear complex were bridged by a phosphate ester, one could expect that the combined electron withdrawing effect of the two Co(III) centres would activate the ester towards attack by coordinated phosphate, resulting in loss of $PNP^-$.




15 16

Figure 8: Proposed synthesis of pyrophosphate, incorporating a binuclear Co(III) complex.

Some publications have described the synthesis of ATP from ADP and an N-phosphorylated polyamine macrocycle in the presence of $Mg^{2+}$, **17**.[15] These experiments found that the yield of ATP increased with an increase in the ratio of the acyl phosphate precursor : ADP to 5 : 1[15a] and with a change of solvent to aqueous dmso.[15b] The increase in ATP production in aqueous dmso was particularly marked: from 19 to 54%. This increase was explained as being due to a decrease in the phosphate hydrolysis reactions that compete with the synthesis of the polyphosphate ester. If **11** were allowed to react in non aqueous solutions or in aqueous solutions of solvents such as dmso the yield of pyrophosphate could be expected to increase for the same reason. Such non-aqueous conditions will, however, move the reaction further away from modelling the reaction which occurs in aqueous biological systems.

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Whilst the yield of pyrophosphate is quite small, routes similar to those just outlined should provide improved yields of polyphosphate derivatives and lead to relatively mild methods of making such anhydride systems compared with current synthetic methods in this area.

## *HYDROLYSIS OF CARBAMOYL PHOSPHATE BY $[N_4Co(OH_2)(OH)]^{2+}$*

### *Postulated Mechanism of Hydrolysis*

Hydrolysis of carbamoyl phosphate is initiated by its coordination to the tamen complex, displacing $H_2O$ as it does so, Figure 9. The resulting binuclear complex, **18**, now has carbamoyl phosphate bound *cis* to coordinated hydroxide and an intramolecular, nucleophilic attack by $OH^-$ on the phosphorus or carbon atoms may occur. In both pathways the final phosphate containing product is the same binuclear species, **20**. However, if coordinated hydroxide ion attacks the phosphorus atom, **18a**, the carbon product is carbamate whereas if it attacks the carbon atom, **18b**, the carbon product will be carbonate. Consequently, if the carbon products of the hydrolysis are identified then the route by which hydrolysis occurs can be established.

Experiments described below investigated the identity of the phosphorus and carbon products of the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$ by $[tamenCo(OH_2)(OH)]^{2+}$ at pH 7.5 and 25 °C. $^{18}O$ tracer experiments were used to confirm the site of attack of Co(III) coordinated hydroxide. The results from these two complementary techniques were uses to identify the route taken by the hydrolysis reaction.

### *Syntheses*

The complexes $[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$, $[tamenCo(CF_3SO_3)_2]CF_3SO_3$, $[(NH_3)_5Co(OPO_3)]$, $[(NH_3)_5Co(OCONH_2)](ClO_4)_2$, and $[(NH_3)_5Co(OCO_2)]CF_3SO_3$ were synthesised by previously established methods.[6] $[tamenCo(CF_3SO_3)_2]CF_3SO_3$

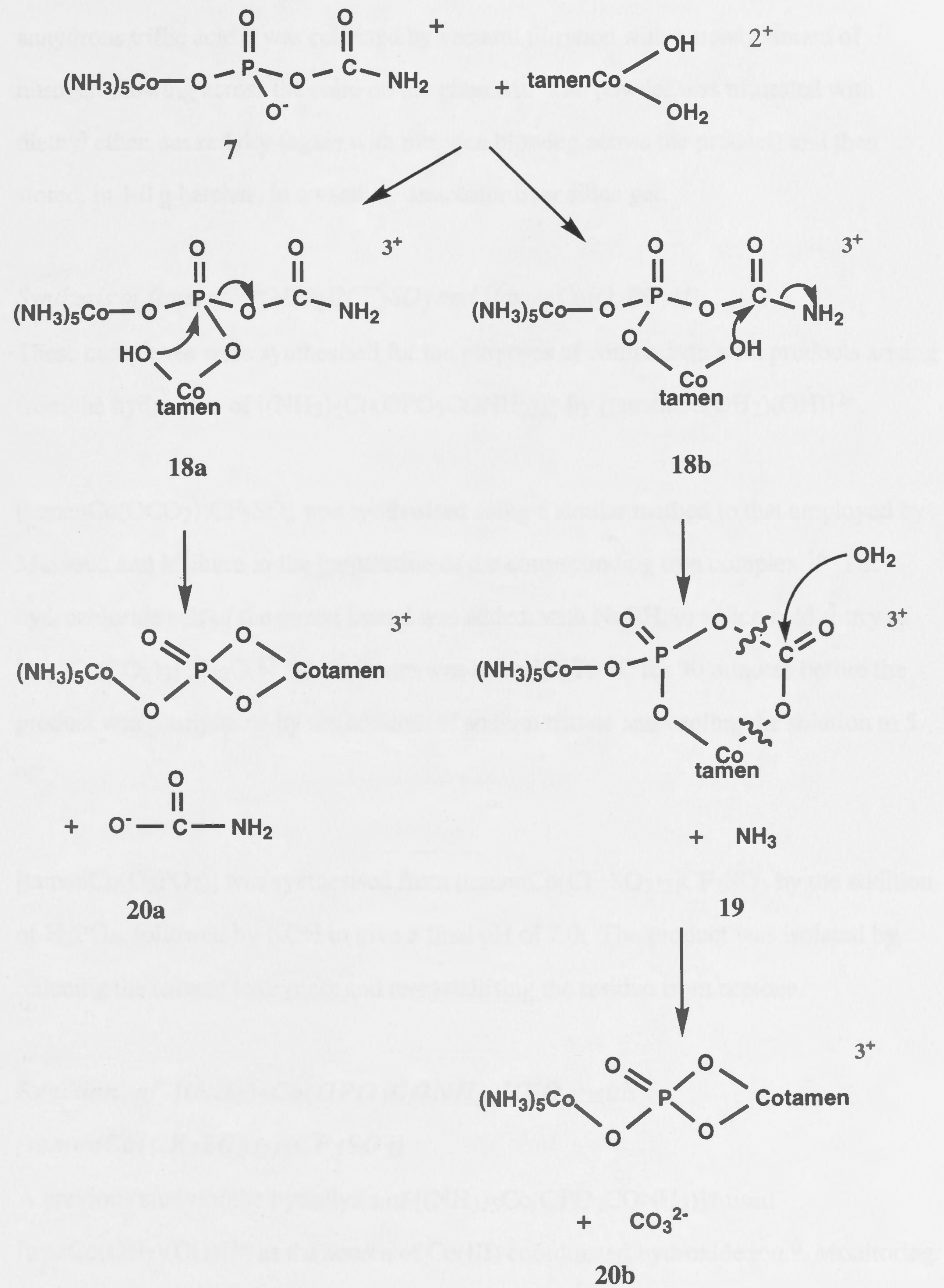



Figure 9: Hydrolysis of coordinated carbamoyl phosphate by $[tamenCo(OH)(OH_2)]^{2+}$ can o by nucleophilic attack at the phosphorus centre to form the products **20a**, or at the carbon centre to form the products **20b**.

proved to be a very hygroscopic material and once it had been precipitated from anhydrous triflic acid it was collected by vacuum filtration with a steady stream of nitrogen blowing across the solid on the glass frit. The powder was triturated with diethyl ether, sucked dry (again with nitrogen blowing across the product) and then stored, in 1.0 g batches, in a vacuum desiccator over silica gel.

*Synthesis of [tamenCo(OCO$_2$)]CF$_3$SO$_3$ and [tamenCo(O$_2$PO$_2$)]*

These complexes were synthesised for the purposes of comparison with products arising from the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$ by $[tamenCo(OH_2)(OH)]^{2+}$.

$[tamenCo(OCO_2)]CF_3SO_3$ was synthesised using a similar method to that employed by Massoud and Milburn in the preparation of the corresponding trpn complex.[16] The hydrochloride salt of the tamen ligand was added, with NaOH, to an ice cold slurry of $Na_3[Co(CO_3)_3].3H_2O$.[17] The mixture was heated to 70 $^oC$ for 90 minutes before the product was precipitated by the addition of sodium triflate and cooling the solution to 5 $^oC$.

$[tamenCo(O_2PO_2)]$ was synthesised from $[tamenCo(CF_3SO_3)_2]CF_3SO_3$ by the addition of $H_3PO_4$, followed by KOH to give a final pH of 7.0. The product was isolated by reducing the solvent to dryness and recrystallising the residue from acetone.

***Reaction of [(NH$_3$)$_5$Co(OPO$_3$CONH$_2$)]ClO$_4$ with [tamenCo(CF$_3$SO$_3$)$_2$](CF$_3$SO$_3$)***

A previous study of the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$ used $[trpnCo(OH_2)(OH)]^{2+}$ as the source of Co(III) coordinated hydroxide ion.[6] Monitoring the reaction by $^{31}P$ nmr spectrometry was complicated by the instability of the trpn complex. Small quantities of Co(II) were produced during the reaction, which caused broadening of the signals of the spectra, necessitating the quenching of samples of the reaction mixture with KCN and Co(II) as the reaction progressed as previously outlined.

In the present experiments however, the more redox stable complex $[tamenCo(OH_2)(OH)]^{2+}$ was used in the hydrolytic experiments without the need to quench with $CN^-$.

$[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$ (0.3 M) was dissolved in a Bis-Tris buffer solution (1.0 M, pH 7.5, 20% $D_2O$). $[tamenCo(CF_3SO_3)_2](CF_3SO_3)$ (0.6 M), dissolved in an equal volume of the Bis-Tris buffer, was added to this solution and the progress of the resulting reaction was monitored by $^{31}P$ nmr spectrometry, Figure 10.

*Identification of Phosphate Products from the Hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$*

The signal due to $[(NH_3)_5Co(OPO_3CONH_2)]^+$ appeared at 11.2 ppm relative to triethylphosphate. Once the tamen complex was added to the reaction mixture this signal became very broad and rapidly decreased in size. This indicated rapid binding and reaction between $[(NH_3)_5Co(OPO_3CONH_2)]^+$ and $[tamenCo(OH_2)(OH)]^{2+}$ and relatively rapid association and dissociation of the components. This change was accompanied by the appearance of two signals at 32.1 and 31.9 ppm; by the end of the reaction (28 minutes) only these peaks remained. Comparison of the nmr spectra with those of the previous study[6] and application of the 'additivity rules'[18] identifies these species as the binuclear complexes **20**. The appearance of two signals for the product is due to the two diastereoisomers of the tamen complex.[12] For the purposes of comparison, the $^{31}P$ nmr spectrum of a solution of $[(NH_3)_5Co(OPO_3)]$ (0.15 M) and $[tamenCo(CF_3SO_3)_2](CF_3SO_3)$ (0.3 M) in Bis-Tris buffer (1.0 M, pH 7.5, 20% $D_2O$) exhibited the same pair of signals at ~ 32 ppm whilst $[tamenCo(O_2PO_2)]$, in a solution of the same buffer, generated a broad signal at 24.9 ppm, characteristic of chelated phosphate.[19] These data support the identification of **20** as the phosphate containing product of the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$.

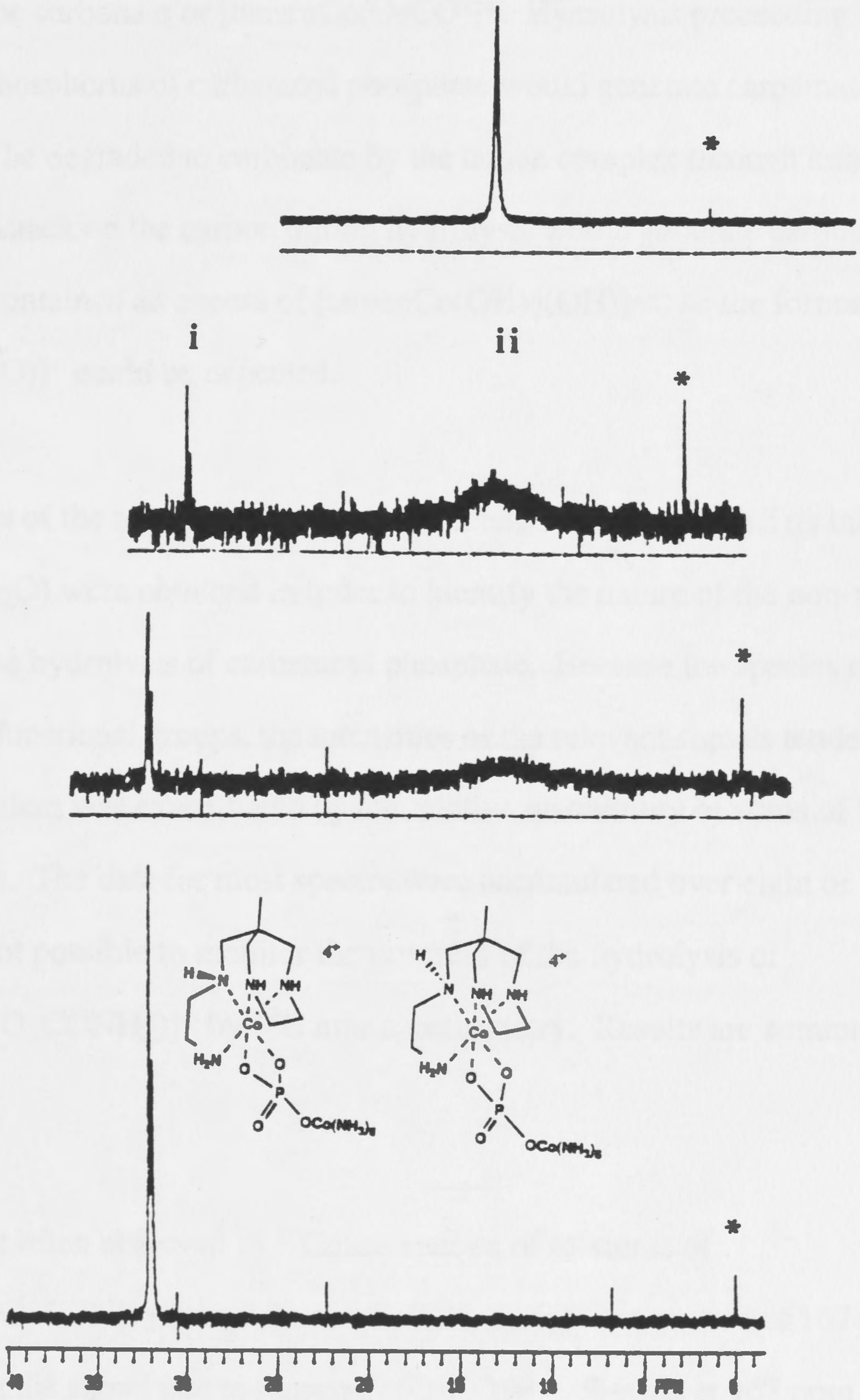



Figure 10: $^{31}$P nmr spectra acquired during hydrolysis of Co(III)-coordinated carbamoyl phosphate by $[tamenCo(OH)(OH_2)]^{2+}$. The complexes were dissolved in a Bis-Tris buffer solution (1.0 M, pH 7.5, 20% $D_2O$, *TEP as internal standard) and spectra acquired at 0, 7, 21 and 40 minutes. **i: 24** (isomers depicted above). **ii:** $[(NH_3)_5Co(OPO_3CONH_2)]^+$.

*Identification of Carbon products from the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$*

The non-phosphate final product from the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$ is most likely to be carbonate or $[tamenCo(O_2CO)]^+$. Hydrolysis proceeding by hydroxide attack on the phosphorus of carbamoyl phosphate would generate carbamate, $NH_2CO_2^-$, and this would be degraded to carbonate by the tamen complex through known chemistry.[31] Attack on the carbon during hydrolysis would generate carbonate directly. The reactions contained an excess of $[tamenCo(OH)_2(OH)]^{2+}$, so the formation of some $[tamenCo(O_2CO)]^+$ could be expected.

$^{13}C$ nmr spectra of the reaction mixture and of related species in Bis Tris buffer (1.0 M, pH 7.5, 20% $D_2O$) were obtained in order to identify the nature of the non-phosphate product(s) of the hydrolysis of carbamoyl phosphate. Because the species of interest were carbonyl functional groups, the intensities of the relevant signals tended to be quite low. This problem was exacerbated by the relative insolubility of some of the $^+1$ charged species. The data for most spectra were accumulated over eight or more hours, and so it was not possible to monitor the progress of the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$ by $^{13}C$ nmr spectrometry. Results are summarised in Table 2.

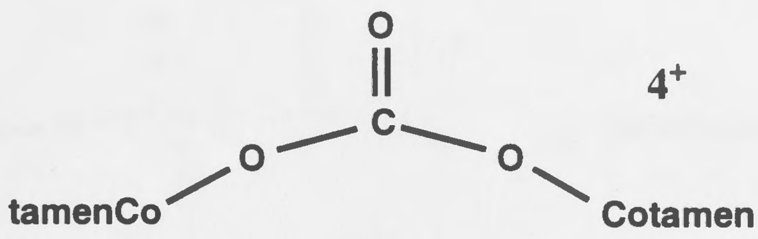
The signal most often observed in $^{13}C$ nmr spectra of mixtures of $[(NH_3)_5Co(OPO_3CONH_2)]^+$ and $[tamenCo(OH_2)(OH)]^{2+}$ occurred at 167 ppm, concurrent with the signal due to $[tamenCo(O_2CO)]^{2+}$. Signals at 167 ppm were also evident in a number of other spectra, implying that this species was more stable than others. However, signals due to other carbonylic species also occurred very close to this chemical shift, particularly uncoordinated bicarbonate and $[(NH_3)_5Co(OCO_2)]^+$. Moreover, the signal to noise ratios of all the carbonyl peaks in the spectra were fairly low and the quality of the signal fairly poor[20] so an absolute assignment of the $^{13}C$ nmr

***Table 2: Chemical shifts of carbonyl moieties of compounds relevant to the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$***

| Species[a] | Chemical Shift, ppm |
|---|---|
| $NaDCO_3$ | 166 |
| $Na_2CO_3$ | 172 |
| $NaOCN$ | 129 |
| $NaDCO_3$ + $[tamenCo(OH_2)(OH)]^{2+}$ | 165 |
| $NaOCN$ + $[tamenCo(OH_2)(OH)]^{2+}$ | 167, 165, 162, 131 (br) |
| $[(NH_3)_5Co(OPO_3CONH_2)]^+$ | 157 |
| $[(NH_3)_5Co(OCONH_2)]^+$ | too insoluble[c] |
| $[(NH_3)_5Co(OCO_2)]^+$ | 168 |
| $[tamenCo(O_2CO)]^+$ | 167 |
| $[(NH_3)_5Co(OPO_3CONH_2)]^+$ + $[tamenCo(OH_2)(OH)]^{2+}$ | 175,[b] 167, 158[b] |
| $[(NH_3)_5Co(OCO_2)]^+$ + $[tamenCo(OH_2)(OH)]^{2+}$ | 175,[b] 167 |
| $[tamenCo(OH_2)(OH)]^{2+}$ | none |

[a] Complexes dissolved in Bis Tris buffer (1.0 M, pH 7.5, 20% $D_2O$) with dioxane as internal standard. [b] Only observed in some samples. [c] Complex as $ClO_4^-$ salt.

signals generated by products of the hydrolysis of carbamoyl phosphate could not be made unequivocally. The signals at 158 and 175 ppm were not observed in any of the samples of the hydrolysis products. This variability was not related to the concentrations of the reagents, since they were kept constant for the purposes of comparison. It is likely that they are due to the presence of transient species in the reaction mixture which dissociate during the long accumulation time required to obtain a spectrum. The signal at 158 ppm is most likely to be due to residual $[(NH_3)_5Co(OPO_3CONH_2)]^+$, whilst that at 175 ppm probably arises from a binuclear species such as **21**.



**21**

A signal also occurs at 175 ppm in spectra of mixtures of $[(NH_3)_5Co(OCO_2)]^+$ and $[tamenCo(OH_2)(OH)]^{2+}$, providing support for this proposition. It is most likely, given its apparent stability and the overlap of its $^{13}C$ nmr signals with those of the hydrolysis reaction, that $[tamenCo(O_2CO)]^+$ is the major non-phosphate product from the hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$. However, this information does not proffer a means of discriminating between hydroxide attack at phosphorus or at carbon of carbamoyl phosphate. A tracer study using $^{18}O$ was required.

### *$^{18}O$ Tracer Study of the Hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]^+$*

If the two mechanisms of hydrolysis are re-examined and the tamen complex is replaced by $[tamenCo(^{18}OH_2)(^{18}OH)]^{2+}$, it is possible to trace the label from substrate to eventual products, Figure 11. Attack of coordinated $^{18}OH^-$ at the phosphorus atom would put $^{18}O$ in the phosphate group, **22a**, and the $^{31}P$ nmr signal of the product would show a satellite peak due to the incorporated labelled oxygen. If however, $^{18}OH^-$ attacked the carbon atom of carbamoyl phosphate, **22b**, the label would end up on the carbonate fragment and not on phosphate. To establish which of these two routes occurs, the following experiment was effected:

$[tamenCo(CF_3SO_3)_2]CF_3SO_3$ (0.3 M) was dissolved in each of two solutions: $H_2O$ and 24% $^{18}O$-labelled water which had been buffered to a pH of 7.5 with Bis Tris buffer.[21] $[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$ (0.15 M) was added to each of these solutions and the resulting mixtures left to stand at 25 °C for 4 hours. A high resolution $^{31}P$ nmr spectrum of each of these samples was acquired. The signal in the spectrum of the labelled sample had a single satellite peak 14 Hz (ppm) upfield from the main peak, Figure 12. The

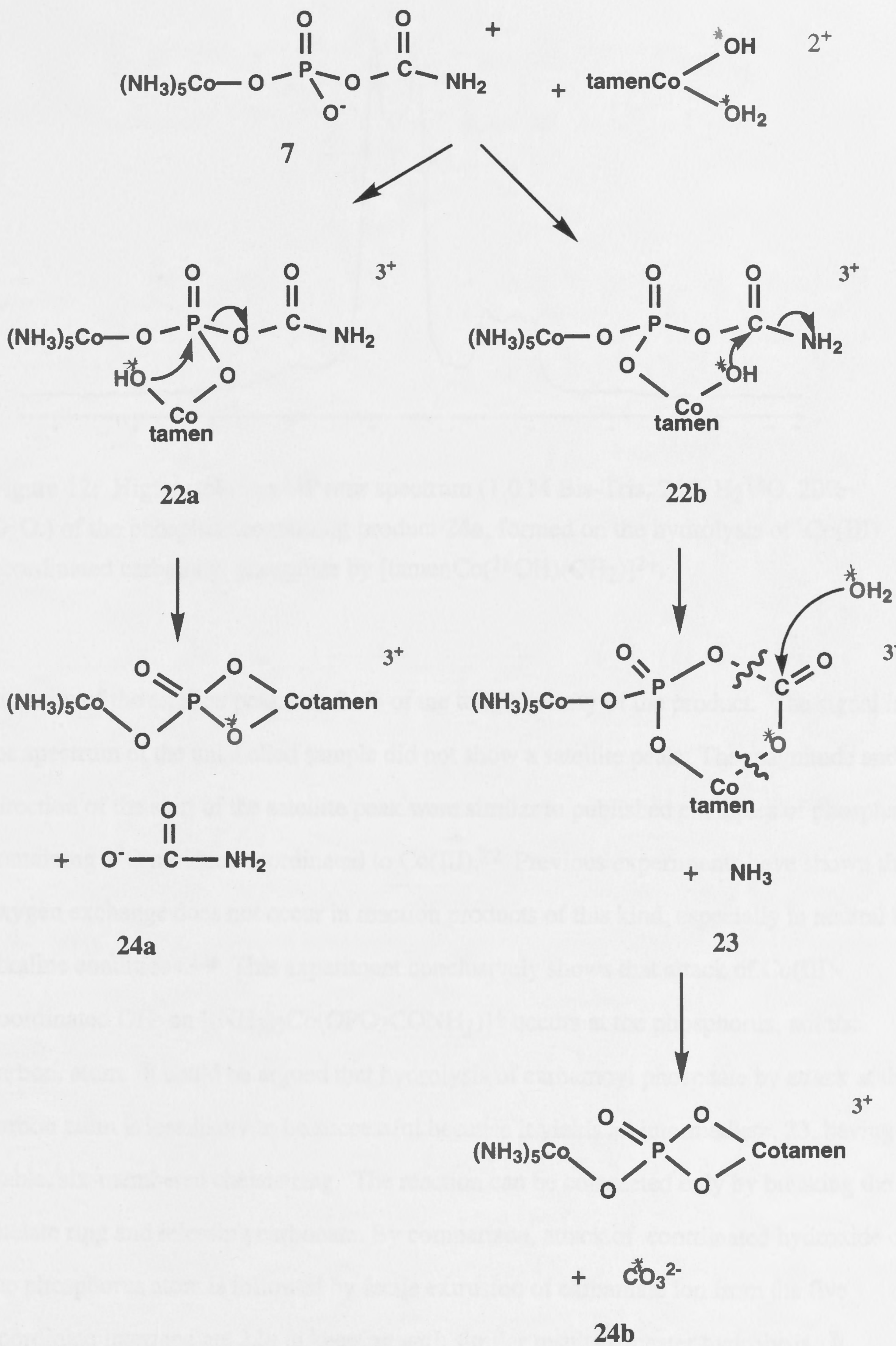



Figure 11: Products of an $^{18}O$ tracer experiment involving the hydrolysis of carbamoyl phosphate by $[tamenCo(\overset{*}{O}H)(\overset{*}{O}H_2)]^{2+}$ ($\overset{*}{O} = {}^{18}O$).

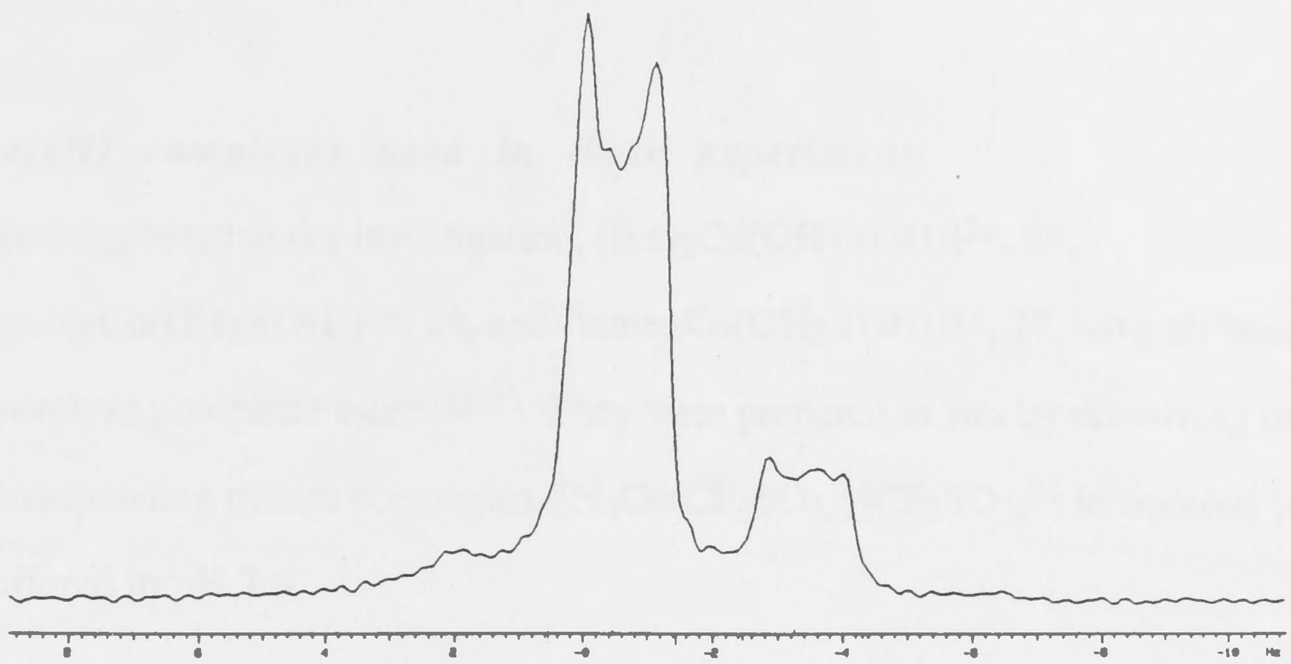


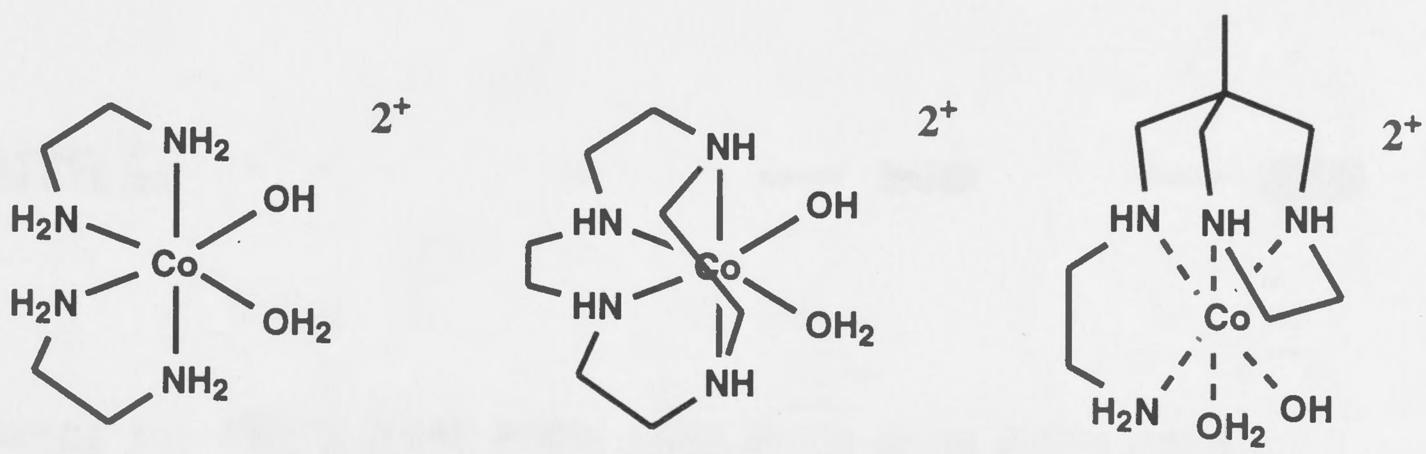
Figure 12: High resolution $^{31}P$ nmr spectrum (1.0 M Bis-Tris, 24% $H_2{}^{18}O$, 20% $D_2O$.) of the phosphate containing product **24a**, formed on the hydrolysis of \Co(III) coordinated carbamoyl phosphate by $[tamenCo(^{18}OH)(OH_2)]^{2+}$.

intensity of the satellite peak was 21% of the total intensity of the product. The signal in the spectrum of the unlabelled sample did not show a satellite peak. The magnitude and direction of the shift of the satellite peak were similar to published examples of phosphate containing $^{18}O$ moieties coordinated to Co(III).[22] Previous experiments have shown that oxygen exchange does not occur in reaction products of this kind, especially in neutral to alkaline conditions.[22a] This experiment conclusively shows that attack of Co(III)-coordinated $OH^-$ on $[(NH_3)_5Co(OPO_3CONH_2)]^+$ occurs at the phosphorus, not the carbon, atom. It could be argued that hydrolysis of carbamoyl phosphate by attack at the carbon atom is less likely to be successful because it yields an intermediate, **23**, having a stable, six-membered chelate ring. The reaction can be completed only by breaking the chelate ring and releasing carbonate. By comparison, attack of coordinated hydroxide on the phosphorus atom is followed by facile extrusion of carbamate ion from the five coordinate intermediate **22a** in keeping with similar results for ester hydrolysis. It follows that the process does not mimic biological activity with transfer of carbamate to the nucleophile and other pathways need to be sought to establish the conditions for such transfers.

## *HYDROLYSIS OF PLASMID DNA*

### *Co(III) complexes used in these experiments*

The complexes under investigation, $[(en)_2Co(OH_2)(OH)]^{2+}$, **25**, $[cyclenCo(OH_2)(OH)]^{2+}$, **26**, and $[tamenCo(OH_2)(OH)]^{2+}$, **27**, have all been shown to hydrolyse phosphate esters.[12,23] They were prepared *in situ* by dissolving their corresponding triflato complexes, $[N_4Co(CF_3SO_3)_2]CF_3SO_3$,[24] in aqueous solutions buffered to pH 7.6.



25 26 27

### *Hydrolysis of Φpuc9*

In a typical experiment, Φpuc9 in Tris buffer (pH 7.6) was treated with complex **25**, **26** or **27**. The resulting solutions were incubated in the dark at 37 °C. Samples were removed at intervals and treated with solutions of KCN and $Co(ClO_4)_2$ to separate the Co(III) complexes from the DNA. The different forms of DNA in the supernatant were then separated by electrophoresis on agarose gel treated with ethidium bromide.[25] A photograph of samples treated in this way appears in Figure 13. Complexes **26** and **27** proved to be capable of converting supercoiled (Form I) DNA into relaxed (Form II) DNA. Under the conditions used in the experiments, **25** did not appear to be capable of hydrolysing Φpuc9. A control experiment, in which the complexes were replaced with $Co(ClO_4)_2$, produced no observable cleavage of DNA so traces of $Co^{2+}$ did not contribute to the hydrolysis.

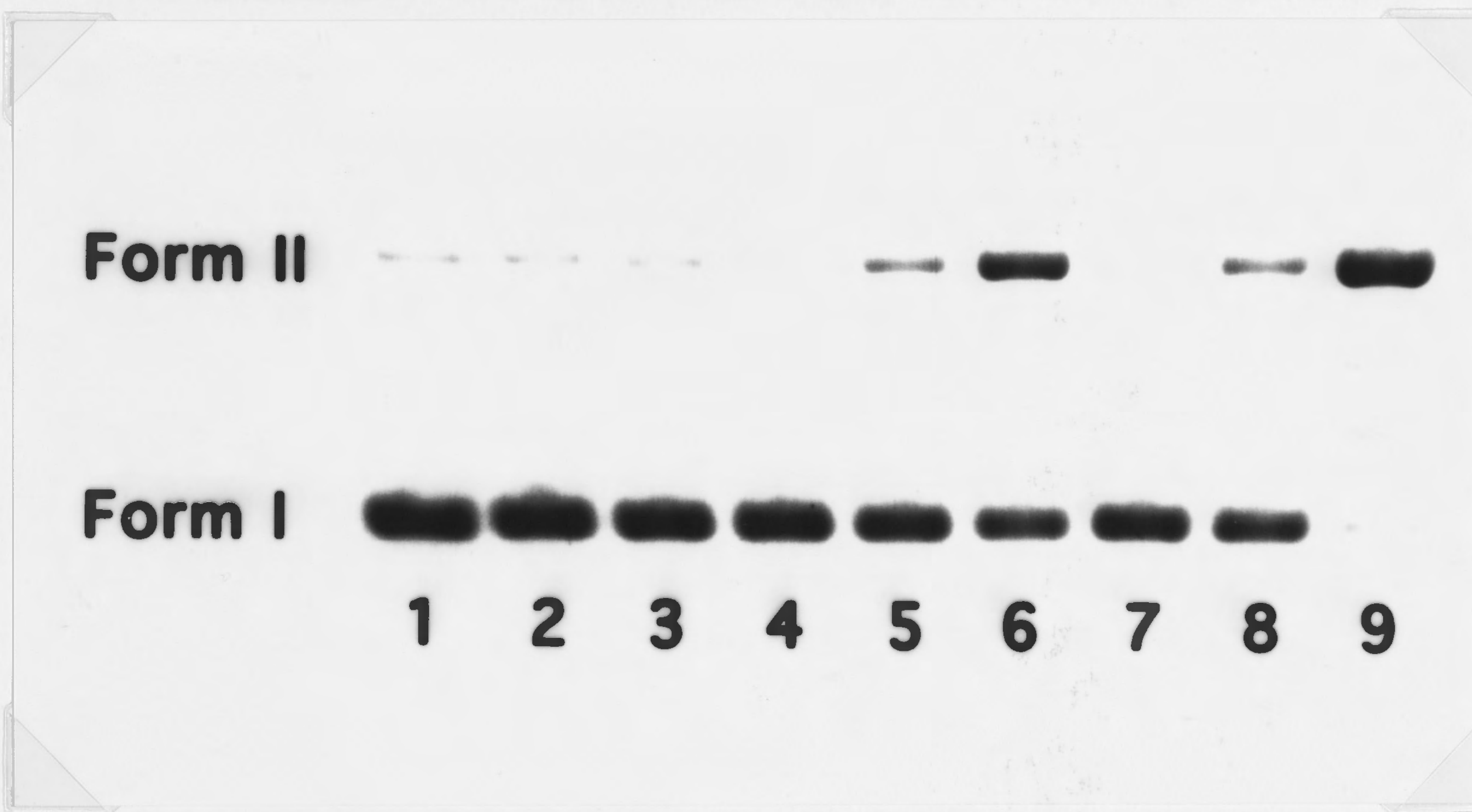



Figure 13: Hydrolysis of puc9 by complexes **25**, **26** and **27** at 37 °C. Coordinated cobalt is removed from the DNA by treating each reaction with excess KCN and a trace of Co(II) proir to loading on the gel. Lanes 1-3, **25** after 0 min, 100 min and 6 h respectively; lanes 4-6, **26** after 0 min, 100 min and 6 h respectively; lanes 7-9, **27** after 0 min, 100 min and 6 h respectively.

Crude values for $k_{obs}$ were obtained by measurement of the changing intensities of bands of supercoiled and/or relaxed DNA in the electrophoresis gels.[26] The rate of increase in relaxed DNA and the rate of decrease of supercoiled DNA yielded pseudo first-order rate constants of 8 x $10^{-6}$, 1 x $10^{-5}$ and 5 x $10^{-5}$ $s^{-1}$ for hydrolysis at 37 °C due to **25**, **26** and **27** respectively.

### *Mechanism of the hydrolysis of DNA by Co(III) complexes*

The mechanism by which the complexes hydrolyse DNA may be postulated after consideration of the mechanism by which these complexes have hydrolysed other, simpler, phosphate diesters.[12,27]

Some recent work in this laboratory focused on the hydrolysis of another diester, cAMP **28**, by a number of hydroxo aqua cobalt(III) complexes.[12] In this instance there are two possible P-O bonds which might cleave upon hydrolysis to generate the 3'- or 5'- AMP complexes, **29a** and **29b**, Figure 14. The ratios of these two products were determined for a range of complexes and considerable variation in the regioselectivity of the hydrolysis reaction was found to have occurred, Table 3.

***Table 3: Observed Ratios[a] of 3'- and 5'- AMP formed on reacting[b] cAMP with a 3 to 5-fold excess of $[N_4Co(OH_2)(OH)]^{2+}$.***

| Complex | Ratio of 3'-AMP to 5'-AMP[c] |
|---|---|
| $[trienCo(OH_2)(OH)]^{2+}$ | 4 (c. 5) |
| $[tamenCo(OH_2)(OH)]^{2+}$ | 2 (c. 3) |
| $[trpnCo(OH_2)(OH)]^{2+}$ | 3 (c. 1.5) |
| $[(tn)_2Co(OH_2)(OH)]^{2+}$ | 0.3 (c. 0.25) |

[a] Taken from Wejesekera *et. al.*, *Aust. J. Chem.*, 1992, **45**, 1187. [b] At pH 7, 25 °C.
[c] Values in parentheses corrected for subsequent hydrolysis of monoesters.

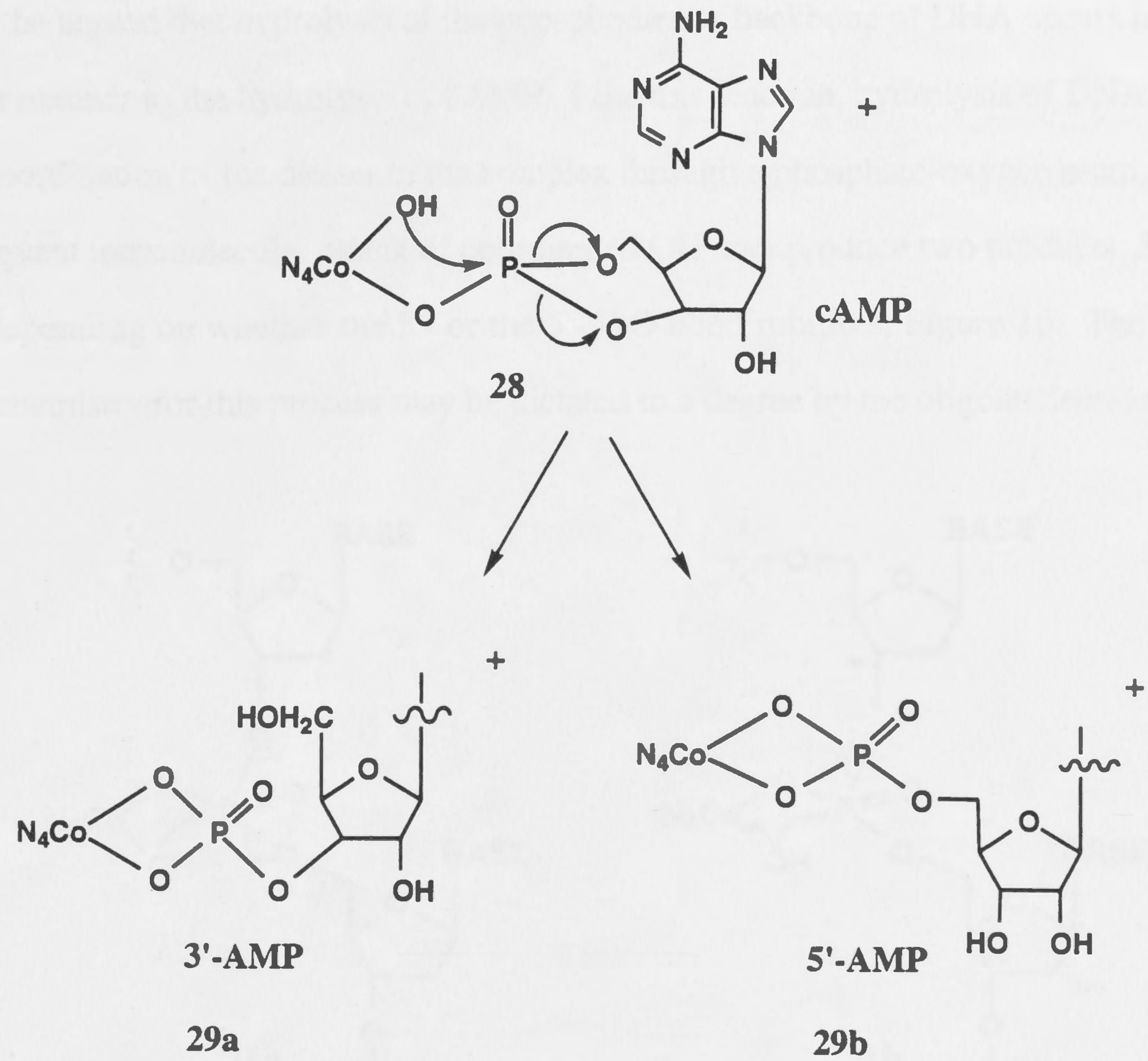



Figure 14: Hydrolysis of c-AMP by $[N_4Co(OH)(OH_2)]^{2+}$.[13]

The dependence of the regioselectivity of hydrolysis upon the Co(III) complex used was explained as a result of steric constraints in the coordination of the diester to the complex, Figure 15.

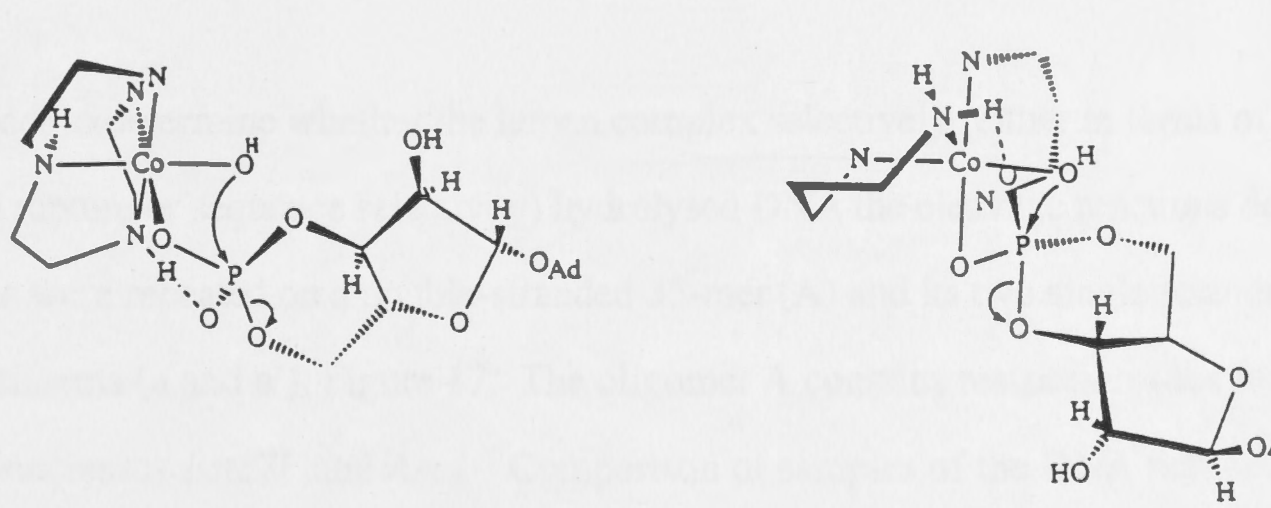


Figure 15: Preferred orientations of trienCo and $tn_2Co$ complexes in the hydrolysis of cAMP. Reproduced from Wijesekera, Hendry and Sargeson, *Aust. J. Chem.*, 1994, **116**, 1121.

It may be argued that hydrolysis of the phosphodiester backbone of DNA occurs in a similar manner to the hydrolysis of cAMP. Like this reaction, hydrolysis of DNA begins with coordination of the diester to the complex through a phosphate-oxygen atom. Subsequent intramolecular attack of coordinated $OH^-$ can produce two products, **30a** and **30b**, depending on whether the 3'- or the 5'- P-O bond ruptures, Figure 16. The stereochemistry for this process may be dictated to a degree by the oligonucleotide.

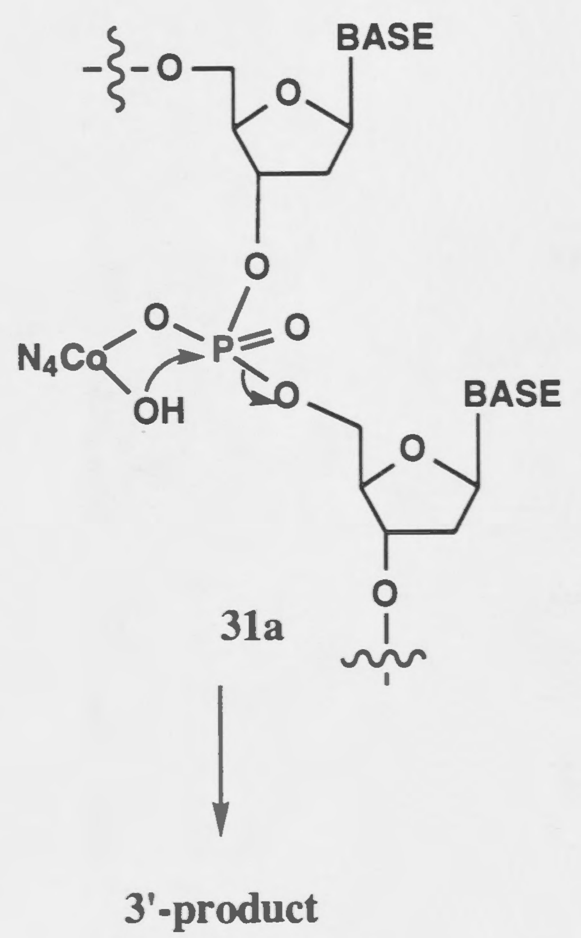
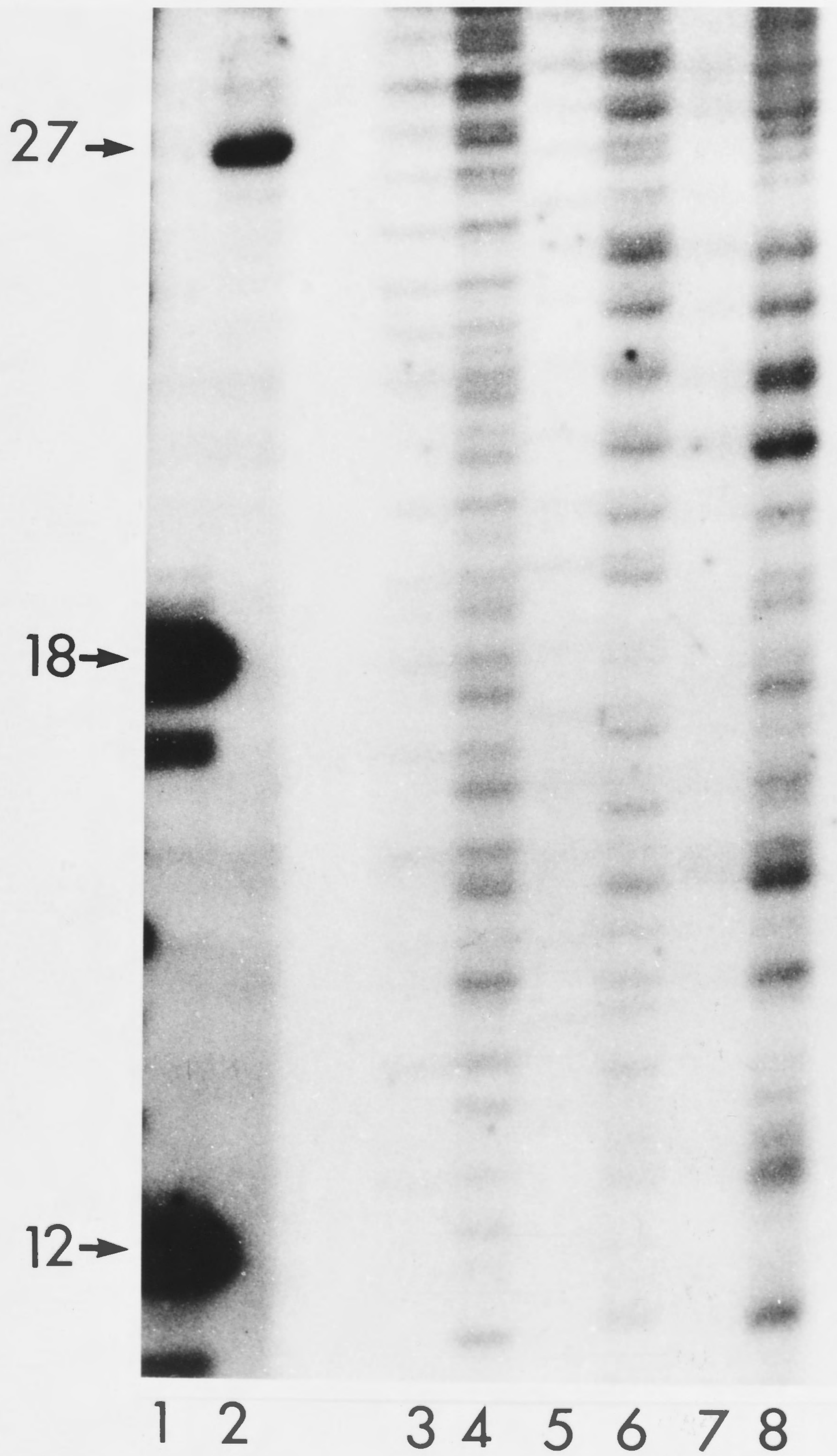



Figure 16: Mechanism of hydrolysis of DNA by $[N_4Co(OH_2)(OH)]^{2+}$.

In order to determine whether the tamen complex selectively (either in terms of 3'/5' P-O bond rupture or sequence selectivity) hydrolysed DNA the cleavage reactions described above were repeated on a double-stranded 35-mer (**A**) and its two single stranded constituents (**a** and **a'**), Figure 17. The oligomer **A** contains restriction sites for the endonucleases *BstEII* and *AspI*. Comparison of samples of the DNA that had been digested with *BstEII* (lane 1), *AspI* (lane 2) and $[tamenCo(OH_2)(OH)]^{2+}$ (lanes 3, 5 and 7) will identify where the complex has clipped the DNA. Figure 17 shows six pairs of bands in digests with the tamen complex that lie between the 18-mer and 12-mer




*AspI* *BstEII*

A: a: 5'-CTAGACTTAGTCCTGAGGGTGACCTTAAGAGATCT-3'
A: a': 3'-GATCTGAATCAGGACTCCCACTGGAATTCTCTAGA-5'

Figure 17: Hydrolytic oligonucleotide cleavage by **27**.

digestion products of *BstEII*. Additionally, between the 27-mer product of *AspI* and the 18-mer product of *BstEII* there are another nine pairs of bands, so DNA hydrolysis by **27** occurred non-specifically with respect to nucleotide sequence.

The hydrolysis products are present in pairs in the electrophoresis gel because they were 5'- end-labelled following digestion with **27**. This is a standard technique which places a phosphate group on the 5'- end of a strand of DNA. The hydrolysis products which already bear a phosphate at the 5'- end (**30b**) will be unchanged by the treatment but the hydrolysis products which bear a phosphate moiety at the 3'-end (**30a**) will gain an additional phosphate group at the 5'-end of the oligomer. In the electrophoresis gel, the more mobile band of each pair will be the doubly phosphorylated oligonucleotide because it has a greater negative charge. The ratio of intensities of the pair of bands will be the ratio of the 3'-P : 5'-P hydrolysis products, **30a** : **30b**. For single-stranded DNA the ratio of 3'-P : 5'-P was approximately 4 : 1 whilst that for double-stranded DNA was approximately 2 : 1. This selectivity is similar in magnitude to and directed towards the same products (3'-P) as was the case for hydrolysis of cAMP by complexes, including **27**, Table 3. It is most likely that the steric constraints that affect the selectivity of hydrolysis of cAMP by hydroxo aqua cobalt(III) complexes also play a part in the selectivity of hydrolysis of DNA by those same complexes.

Although there is selectivity for 3'- versus 5'- hydrolysis, there is little sequence selectivity. Complexes such as these would need to be tethered to a sequence selective footprint to achieve specificity in this respect. Synthesis of such reagents is now in progress.

## Concluding Remarks

The studies described in this chapter are linked mechanistically. In each instance the chemistry has been made available by coordination of a phosphate ester cis to a coordinated nucleophile. The phosphorus atom of the ester becomes more electrophilic on coordination and hence more susceptible to nucleophilic attack.[2] Addition of a nucleophile, such as hydroxide ion, has proven to be particularly facile when it occurs via an intramolecular pathway, such as when the two species are coordinated to a metal ion.[2] The reaction is completed by elimination of the ester moiety (in these studies $PNPP^-$, carbamate and the DNA backbone).

Studies of the synthesis and hydrolysis of phosphate derivatives provide information about the processes which result in the biosynthesis and degradation of such species. They may also result in the development of new tools for molecular biology and new synthetic strategies for chemistry in general.

## Experimental

### *INSTRUMENTS, REAGENTS AND ANALYSES*

Nuclear magnetic resonance spectra of the complexes dissolved in $D_2O$, 20% $D_2O$ in $H_2O$, or 0.1 M DCl were acquired using a Bruker CXP-200 ($^{31}P$ nmr spectra of pyrophosphate syntheses), Varian VXR-300 ($^{31}P$, $^{13}C$, $^{1}H$ nmr spectra of pyrophosphate syntheses) or a Varian Gemini 300 NMR spectrophotometer ($^{31}P$, $^{13}C$, $^{1}H$ nmr spectra of carbamoyl phosphate and DNA hydrolysis). Chemical shifts in $^{1}H$ nmr spectra are reported relative to sodium trimethylsilylpropane sulfonate (NaTPS), 0.00ppm. Chemical shifts in $^{13}C$ nmr spectra are reported relative to dioxane, 67.4 ppm. Chemical shifts in $^{31}P$ nmr spectra are reported relative to triethylphosphate, -0.6 ppm. Visible spectra were obtained using a Cary 118C spectrophotometer. pH measurements were made with a Radiometer PHM 26 pH meter calibrated using standard buffers and G202C glass electrodes. The percentage enrichment of $^{18}O$-labelled water was determined by mass spectroscopy. The water was vacuum distilled before use. Elemental microanalyses were performed by the ANU Microanalytical Service. Experiments in DNA hydrolysis by Co(III) complexes were performed in collaboration with Nicholas Dixon, John Lambert and Shadi Moghaddas, also of the Research School of Chemistry, ANU.

Most solvents and basic chemicals used for syntheses were analytical reagent grade. Ion exchange chromatography was performed with analytical grade Dowex 50Wx2 ($H^+$ form, 200 - 400 mesh, Bio-Rad), Dowex AG 50Wx8 ($H^+$ form, 50 - 100 mesh, Bio Rad), SP Sephadex C25 ($Na^+$ form, Pharmacia) or S Sepharose ($Na^+$ form, 45 - 165 μm wet, Pharmacia). Complexes present in the collected eluents were recovered by evaporation (~ 20 τ) on a Büchi rotary evaporator, with a water bath temperature of less than 40 °C.

## *SYNTHESES*

The complexes $[tamenCo(CF_3SO_3)_2]CF_3SO_3$, $[cyclenCo(CF_3SO_3)_2]CF_3SO_3$ and $[(en)_2Co(CF_3SO_3)_2]CF_3SO_3$ were prepared using previously established syntheses, by the addition of the corresponding dinitrito or dichloro complexes to anhydrous triflic acid.[6] $[(tn)_2Co(O_2PO_2)]$[28] and $[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$[12] were also prepared by established syntheses.

### *Synthesis of $[(tn)_2Co(OPO_2OH)(OPO_2OHC_6H_4NO_2)]$*

Disodium p-nitrophenylphosphate (0.742 g, 2 mmol) was dissolved in water (50 $cm^3$) and washed through a bed of AG 50Wx8 resin (50 $cm^3$ of resin, ~ 3.0 x 6.5 cm). The pH of the eluent was monitored with pH paper and the acid ($H_2PNPP$) collected over the pH range 1.5 - 5.5. The solvent was removed by rotary evaporation. $[tn_2Co(O_2PO_2)]$

(0.604 g, 2 mmol) was added to the resulting white solid and a minimum volume of water (~ 4 $cm^3$) added to dissolve both solids (the chelate phosphate is quite insoluble, so the solubility of the complex indicated that the chelate ring had opened). After 5 minutes at 25 °C, the reaction was quenched by precipitating the product with ethanol (~ 30 $cm^3$). The lilac coloured powder that precipitates was collected by vacuum filtration, washed with ethanol and diethyl ether before being dried under vacuum and over $P_2O_5$ for 24 hours (1.00 g, 96%). Analysis calculated for $[CoC_{12}H_{26}N_5P_2O_{10}].0.5H_2O$: Co, 11.11; C, 27.18; H, 5.13; N, 13.21; P, 11.86. Found: C, 25.6; H, 5.1; N, 13.0; P, 11.8. $^1H$ nmr ($D_2O$): δ 8.28, 7.40 (AXq, 4H, $C_6H_4$); 3.4 - 5.8 (br, 8H, tn-$NH_2$); 1.7 - 3.0 (br, 12H, tn-$CH_2$). $^{13}C$ nmr ($D_2O$): δ 118.2, 121.4, 121.5, 126.7, 126.8, 130.4 ($C_6H_4$); 25.8, 25.9, 38.9, 38.9, 39.1, 39.2 (tn-$CH_2$). $^{31}P$ nmr ($D_2O/H_2O$): δ 4.8, ($PNPP^{2-}$); 11.2, ($PO_4^{3-}$).

***Synthesis of $[(tn)_2Co(P_2O_7)]^-$***

*($^{31}P$ nmr spectrometric experiment)*

A solution of **11** (0.05 M) in water, HCl or buffer (0.5 M, 30 $cm^3$) was made and left to stand at 25 °C. Samples (5 $cm^3$) of this solution were 'quenched' (see below) at intervals (up to 28 days) and $D_2O$ (1 $cm^3$) added to them for $^{31}P$ nmr spectrometry. The buffer solutions were MES (pH 5.45 and 6.22) and HEPES (pH 7.50).

***Procedure for 'Quenching' a Reaction with $Co^{2+}$ and $CN^-$***

Samples of the reaction mixture (0.05 M in complex) were quenched by the addition of KCN (0.5 g) and a trace (a couple of small crystals) of $Co(ClO_4)_2.6H_2O$. The mixture was allowed to react for 5 minutes and then centrifuged to remove any solid material. $D_2O$ ( 1 $cm^3$) was added to the supernatant (having a pH of 9.5) for $^{31}P$ nmr spectrometry. Spectral assignments of $H_2PNPP$, $NaH_2PO_4$, $Na_4P_2O_7$ were confirmed by adding authentic samples to the nmr solutions: phosphate, 4.65 ppm; p-nitrophenylphosphate, 1.30 ppm; pyrophosphate, -3.70 ppm.

### *Synthesis of [tamenCo($O_2CO$)]$CF_3SO_3$*

This complex was synthesised in a similar manner to the corresponding trpn complex.[29] A solution of the tamen ligand (as its hydrochloride salt, 3.32 g) and NaOH (0.40 g) in water (15 $cm^3$) was added to an ice cold slurry of $Na_3[Co(CO_3)_3].3H_2O$[30] (3.60 g in 25 $cm^3$ water). The stirring solution was warmed to 70 $^oC$ and heated at this temperature for 1.5 hours. Sodium triflate (2 g) was added to the hot solution and it was then chilled at 5 $^oC$ for ~ 4 days. A sticky, dark, purplish material was collected, dissolved in ethanol and then left standing to be reduced almost to dryness, producing a very dark pink microcrystalline solid. This was collected by vacuum filtration, washed with acetone and diethyl ether and dried under vacuum (2.62 g, 63%). Analysis calculated for $[CoC_{11}H_{22}N_4O_6SF_3].H_2O$: Co, 12.48; C, 27.97; H, 5.12; N, 11.86; S, 6.81; F, 12.07. Found: C, 27.7; H, 5.3; N, 11.6. $^{13}C$ nmr ($D_2O$): δ 167.7 (C=O), 62.3, 61.8, 58.8, 55.8, 54.7, 43.6, 43.3, 39.5 ($C_q$ and tamen, 7 x $CH_2$), 21.1 ($CH_3$).

### *Synthesis of [tamenCo($O_2PO_2$)]*

[tamenCo$(CF_3SO_3)_2$]$CF_3SO_3$ (0.35 g) was dissolved in water (0.6 $cm^3$) in a small rotary evaporation flask. $H_3PO_4$ (85%, 0.012 $cm^3$ diluted to 6 $cm^3$ with water) was added to the resulting solution. The pH of the solution was then adjusted to 7 with 2 M KOH and the flask put on the rotary evaporator and warmed at 60 $^oC$ for 20 minutes. The temperature of the water bath was decreased to 30 $^oC$ and the solution reduced to dryness. The purple residue was recrystallised from acetone to give a purple powder that was collected by vacuum filtration, washed with diethyl ether and dried over silica gel (0.12 g, 69%).

Analysis calculated for $[CoC_9H_{22}N_4PO_4]$: Co, 19.32; C, 31.18; H, 6.52; N, 16.47; P, 9.10. Found: C, 31.3; H, 6.6; N, 16.3. $^{13}C$ nmr ($D_2O$): δ 167.9, (C=O), 62.4, 61.9, 57.0, 55.9, 54.9, 43.8, 43.5, 39.8, ($C_q$ and tamen 7 x $CH_2$), 21.4 ($CH_3$). $^{31}P$ nmr ($D_2O$): δ 24.9.

### *Hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$ by $[tamenCo(OH_2)(OH)]^{2+}$ ($^{31}P$ nmr spectrometric experiment)*

$[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$ (0.029 g, 7.5 x $10^{-5}$ moles) was dissolved in Tris buffer solution (0.7 $cm^3$, 1.0 M, pH 7.5, 20% $D_2O$) and a $^{31}P$ nmr spectrum acquired (1 drop of 0.5 M triethyl phosphate as internal standard). $[tamenCo(CF_3SO_3)_2]CF_3SO_3$ (0.104 g, 1.5 x $10^{-4}$ moles), dissolved in the same buffer solution (0.3 $cm^3$) was added to the solution in the nmr tube and $^{31}P$ nmr spectra recorded at intervals until no further change was observed; a $^{13}C$ nmr spectrum was then acquired. This experiment was repeated using HEPES buffer (1.0 M, pH 7.5) in place of Tris buffer, to try to reduce the size of the signals due to the buffer, relative to those of the products, in the $^{13}C$ nmr spectrum, but it made little difference.

### *$^{18}O$ Tracer Study of the Hydrolysis of $[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$ by $[tamenCo(OH_2)(OH)]^{2+}$*

$[(NH_3)_5Co(OPO_3CONH_2)]ClO_4$ (0.029 g, 7.5 x $10^{-5}$ moles) was dissolved in a mixture consisting of $H_2{}^{18}O$ (0.7 $cm^3$) + 1 drop of $D_2O$ + 1 drop triethyl phosphate solution (0.5 M in $D_2O$). $[tamenCo(CF_3SO_3)_2]CF_3SO_3$ (0.104 g, 1.5 x $10^{-4}$ moles) and Tris (0.105 g, 5.0 x $10^{-4}$ moles) were dissolved in 0.3 $cm^3$ of $H_2{}^{18}O$. $D_2O$ and $^{31}P$ nmr spectrum acquired until no further change occurred (~ 35 minutes). A high resolution $^{31}P$ nmr spectrum was then acquired, to see the satellite peak due to labelled oxygen. The proportion of label incorporated into the products was determined by integration of the signals. This experiment was repeated, $H_2O$ replacing $H_2{}^{18}O$ and a high resolution $^{31}P$ nmr spectrum acquired for the purposes of comparison.

### *Hydrolysis of Φpuc9 by $[tamenCo(OH_2)(OH)]^{2+}$ and other complexes*

In a typical experiment, Φpuc9 (32.6 μM) in Tris buffer (10 mM, pH 7.6) was treated with a freshly prepared solution of complex **25**, **26** or **27** (1 mM in 10 mM Tris, pH 7.6). The resulting solutions were incubated in the dark at 37 °C. Samples (35 μL aliquots) were removed at intervals and treated with solutions of KCN (100 mM) and

$Co(ClO_4)_2$ (~ 5 mM) to separate the Co(III) complexes from the DNA. The different forms of DNA in the supernatant were then separated by electrophoresis on a 1% agarose gel/TBE buffer impregnated with 0.5 μg $mL^{-1}$ ethidium bromide.[25] The resulting bands were illuminated with a UV transilluminator and photographed.

***Kinetic analysis of the hydrolysis of Φpuc9 by $[tamenCo(OH_2)(OH)]^{2+}$ and other complexes***

Form I and Form II DNA in electrophoresis gels from the above experiments were made visible with the aid of ethidium bromide and a uv-light box, photographed and the negatives scanned on a UVR scanner set on transmission mode. Band densities for the various time points were measured using the public domain software NIH Image.

***Hydrolysis of Oligonucleotide by $[tamenCo(OH_2)(OH)]^{2+}$***

The oligonucleotides (23 μg $ml^{-1}$) were incubated with $[tamenCo(OH_2)(OH)]^{2+}$ (1 mM) in Tris buffer (20 mM, pH 7.4) for 18 hours at 37 °C in the dark. Control reactions were not treated with $[tamenCo(OH_2)(OH)]^{2+}$. After incubation a portion of each sample was end-labelled directly and electrophoresed on a 20% acrylamide/7M urea sequencing gel.

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# Appendices

## Appendix I: Data from the X-ray crystallographic analysis of $[Co(NH_3)_3(C_6H_9N_2)_4)](ClO_4)_2.2H_2O$

**Table I. Crystallographic Data for $[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2{\cdot}2H_2O$.**

| | |
|---|---|
| chem formula | $C_6H_{22}Cl_2CoN_5O_{14}$ |
| fw | 518.11 |
| cryst system | triclinic |
| spacegroup | $P\overline{1}$ |
| a, Å | 8.682(3) |
| b, Å | 9.519(3) |
| c, Å | 12.432(4) |
| α,° | 102.12(3) |
| β,° | 99.82(3) |
| γ,° | 104.23(2) |
| V, Å$^3$ | 946.6(6) |
| Z | 2 |
| $d_{calcd}$, g cm$^{-3}$ | 1.818 |
| μ[Mo Kα], cm$^{-1}$ | 12.7 |
| T, °C | 22(1) |
| cryst dimens, mm | 0.20 × 0.28 × 0.40 |
| X-radiation | Mo Kα |
| λ, Å | 0.71069 |
| data range, ° in 2θ | 5 - 50 |
| no. unique data | 3330 |
| no. data refined | 2838 [$I > 3\sigma(I)$] |
| no. variables | 281 |
| no. restraints | 24 |
| $R$ | 0.054 |
| $R_w$ | 0.081 |
| $GOF$ | 3.13 |
| $F(000)$ | 531.90 |

$R = \Sigma \, ||F_o| - |F_c|| / \Sigma \, |F_o|$; $R_w = [\Sigma w(|F_o| - |F_c|)^2 / \Sigma \, (wF_o^2)]^{½}$

$GOF = [\Sigma w(|F_o| - |F_c|)^2 / (\text{no. ref - no. var})]^{½}$

**Table II. Atomic coordinates and equivalent isotropic displacement parameters[a] for the non-hydrogen atoms in $[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2 \cdot 2H_2O$**

| | *x/a* | *y/b* | *z/c* | $U_{eq}$ / $U$ |
|---|---|---|---|---|
| Co | 0.71141(7) | 0.26616(7) | 0.15919(5) | 0.0360(3) |
| O(1) | 0.5106(4) | 0.1119(4) | 0.1165(3) | 0.044(1) |
| O(2) | 0.3164(4) | -0.0109(4) | 0.1835(3) | 0.053(1) |
| O(3) | 0.3346(4) | 0.5728(4) | 0.3183(3) | 0.053(2) |
| O(4) | 0.4942(5) | 0.6660(4) | 0.2122(3) | 0.057(2) |
| N(1) | 0.7060(5) | 0.2450(5) | 0.3107(3) | 0.042(2) |
| N(2) | 0.6050(4) | 0.4199(4) | 0.1885(3) | 0.036(1) |
| N(3) | 0.9244(5) | 0.4208(5) | 0.2090(4) | 0.051(2) |
| N(4) | 0.6843(6) | 0.2750(5) | 0.0013(3) | 0.050(2) |
| N(5) | 0.8170(6) | 0.1062(5) | 0.1241(4) | 0.062(2) |
| C(1) | 0.4427(6) | 0.0893(5) | 0.1975(4) | 0.040(2) |
| C(2) | 0.5290(6) | 0.1984(5) | 0.3136(4) | 0.038(2) |
| C(3) | 0.5059(7) | 0.1262(6) | 0.4097(5) | 0.055(2) |
| C(4) | 0.4638(6) | 0.3346(6) | 0.3264(4) | 0.042(2) |
| C(5) | 0.5104(5) | 0.4378(5) | 0.2548(4) | 0.036(2) |
| C(6) | 0.4454(6) | 0.5725(5) | 0.2596(4) | 0.039(2) |
| O(5) | 0.1989(5) | -0.2207(5) | 0.3017(4) | 0.070(2) |
| O(6) | 0.7674(6) | -0.0494(5) | 0.3013(4) | 0.082(2) |
| Cl(1) | 0.1653(2) | 0.2979(2) | -0.0153(1) | 0.0566(6) |
| O(11A)[b] | 0.068(2) | 0.172(2) | -0.091(1) | 0.23(1) |
| O(12A)[b] | 0.3126(9) | 0.363(1) | -0.0282(7) | 0.122(6) |
| O(13A)[b] | 0.1801(9) | 0.278(1) | 0.0973(7) | 0.108(5) |
| O(14A)[b] | 0.075(1) | 0.4117(9) | -0.0108(8) | 0.115(5) |
| O(11B)[c] | 0.169(2) | 0.372(2) | -0.100(1) | 0.083(4) |
| O(12B)[c] | 0.304(2) | 0.225(2) | -0.024(1) | 0.083(4) |
| O(13B)[c] | 0.185(2) | 0.394(2) | 0.083(1) | 0.083(4) |

| | | | | |
|---|---|---|---|---|
| O(14B)[c] | 0.024(3) | 0.177(3) | -0.053(2) | 0.083(4) |
| Cl(2) | 0.8919(2) | 0.7109(2) | 0.4986(1) | 0.0609(6) |
| O(21A)[d] | 0.931(2) | 0.735(2) | 0.614(1) | 0.102(5) |
| O(22A)[d] | 0.729(1) | 0.698(2) | 0.4563(7) | 0.130(6) |
| O(23A)[d] | 0.993(1) | 0.822(1) | 0.4657(9) | 0.154(7) |
| O(24A)[d] | 0.918(2) | 0.572(1) | 0.446(1) | 0.21(1) |
| O(21B)[e] | 0.849(2) | 0.853(2) | 0.503(1) | 0.087(5) |
| O(22B)[e] | 0.765(2) | 0.590(2) | 0.452(2) | 0.087(5) |
| O(23B)[e] | 1.029(2) | 0.703(2) | 0.462(2) | 0.087(5) |
| O(24B)[e] | 0.938(4) | 0.723(4) | 0.626(3) | 0.087(5) |

Footnotes:

a $U_{eq} = \frac{1}{3} \sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j$

b Occupancy 0.695(9)

c Occupancy 0.305(9); one common isotropic displacement factor for all atoms O(1nB).

d Occupancy 0.70(1)

e Occupancy 0.30(1); one common isotropic displacement factor for all atoms O(2nB).

## SUPPLEMENTARY MATERIAL FOR DEPOSITION

$[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2 \cdot 2H_2O$

Table SUP-I. H-atom coordinates.

Table SUP-II. Anisotropic displacement factors.

Table SUP-III. Torsion angles for non-H atoms of cation.

Table SUP-IV. Additional distances, angles and torsion angles

Table SUP-V. Least-squares planes.

Table SUP-VI. Structure factor listing.

**Table SUP-L Atomic coordinates and isotropic displacement parameters for the hydrogen atoms in $[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2 \cdot 2H_2O$**

| | *x/a* | *y/b* | *z/c* | *U* |
|---|---|---|---|---|
| H(1) | 0.2944 | 0.6393 | 0.3183 | 0.070 |
| H(2) | 0.7644 | 0.3295 | 0.3691 | 0.070 |
| H(3) | 0.7524 | 0.1662 | 0.3215 | 0.070 |
| H(4) | 0.6179 | 0.4812 | 0.1530 | 0.070 |
| H(5) | 0.3982 | 0.0965 | 0.4137 | 0.070 |
| H(6) | 0.5492 | 0.0463 | 0.4040 | 0.070 |
| H(7) | 0.5746 | 0.1962 | 0.4729 | 0.070 |
| H(8) | 0.3491 | 0.3033 | 0.3123 | 0.070 |
| H(9) | 0.5082 | 0.3878 | 0.4008 | 0.070 |
| H(10) | 0.9139 | 0.5017 | 0.2472 | 0.070 |
| H(11) | 0.9919 | 0.3911 | 0.2499 | 0.070 |
| H(12) | 0.9594 | 0.4366 | 0.1515 | 0.070 |
| H(13) | 0.6951 | 0.1955 | -0.0388 | 0.070 |
| H(14) | 0.5897 | 0.2824 | -0.0228 | 0.070 |
| H(15) | 0.7563 | 0.3510 | -0.0031 | 0.070 |
| H(16) | 0.9132 | 0.1444 | 0.1175 | 0.070 |
| H(17) | 0.8216 | 0.0627 | 0.1772 | 0.070 |
| H(18) | 0.7617 | 0.0424 | 0.0624 | 0.070 |
| H(19) | 0.1393 | -0.2103 | 0.3270 | 0.100 |
| H(20) | 0.2476 | -0.1496 | 0.2615 | 0.100 |
| H(21) | 0.8042 | -0.0493 | 0.3654 | 0.100 |
| H(22) | 0.6863 | -0.1372 | 0.2500 | 0.100 |

**Table SUP-II. Anisotropic displacement parameters for the non-hydrogen atoms in $[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2 \cdot 2H_2O$**

| | $U_{11}$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{13}$ | $U_{23}$ |
|---|---|---|---|---|---|---|
| Co | .0398(4) | .0338(4) | .0364(4) | .0110(3) | .0113(3) | .0114(3) |
| O(1) | .052(2) | .038(2) | .036(2) | .003(2) | .012(1) | .005(1) |
| O(2) | .053(2) | .044(2) | .053(2) | -.000(2) | .013(2) | .012(2) |
| O(3) | .059(2) | .045(2) | .068(2) | .023(2) | .031(2) | .018(2) |
| O(4) | .062(2) | .055(2) | .071(2) | .026(2) | .029(2) | .035(2) |
| N(1) | .042(2) | .044(2) | .040(2) | .010(2) | .005(2) | .016(2) |
| N(2) | .037(2) | .034(2) | .034(2) | .006(2) | .005(2) | .013(2) |
| N(3) | .043(2) | .054(3) | .060(3) | .013(2) | .015(2) | .021(2) |
| N(4) | .061(3) | .045(2) | .041(2) | .010(2) | .016(2) | .013(2) |
| N(5) | .075(3) | .047(3) | .082(3) | .029(2) | .041(3) | .027(2) |
| C(1) | .042(3) | .032(2) | .044(3) | .007(2) | .008(2) | .012(2) |
| C(2) | .042(3) | .035(2) | .035(2) | .009(2) | .007(2) | .010(2) |
| C(3) | .067(4) | .053(3) | .049(3) | .014(3) | .019(3) | .023(3) |
| C(4) | .049(3) | .042(3) | .038(2) | .014(2) | .015(2) | .010(2) |
| C(5) | .034(2) | .033(2) | .034(2) | .005(2) | .003(2) | .005(2) |
| C(6) | .037(2) | .035(2) | .039(2) | .007(2) | .005(2) | .006(2) |
| O(5) | .079(3) | .063(3) | .098(3) | .039(2) | .050(3) | .039(2) |
| O(6) | .116(4) | .053(3) | .075(3) | .024(3) | .007(3) | .026(2) |
| Cl(1) | .0471(7) | .0517(8) | .0664(9) | .0074(6) | .0079(6) | .0187(7) |
| O(11A) | .14(1) | .127(9) | .30(2) | .037(9) | -.05(1) | -.11(1) |
| O(12A) | .066(5) | .21(1) | .113(6) | .027(6) | .041(4) | .094(7) |
| O(13A) | .079(5) | .176(9) | .113(6) | .044(5) | .045(4) | .105(7) |
| O(14A) | .131(7) | .102(7) | .164(9) | .065(6) | .070(7) | .081(6) |
| Cl(2) | .0527(8) | .068(1) | .0630(9) | .0160(7) | .0109(7) | .0223(7) |
| O(21A) | .072(5) | .18(1) | .043(4) | .027(5) | -.006(3) | .039(5) |
| O(22A) | .073(5) | .23(1) | .078(5) | .062(7) | -.007(4) | .028(7) |
| O(23A) | .17(1) | .133(9) | .152(9) | -.007(8) | .090(8) | .049(7) |
| O(24A) | .33(2) | .099(9) | .20(1) | .11(1) | .07(1) | -.017(8) |

Anisotropic displacement parameters in the form: $-2\pi^2(U_{11}h^2a^{*2} + .... +2U_{12}hka^*b^* + ....)$.

**Table SUP-III. Torsion angles (°) for non-hydrogen atoms of cation of $[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2 \cdot 2H_2O$**

| | | | |
|---|---|---|---|
| N(1)-Co-O(1)-C(1) | 16.0(4) | N(2)-Co-O(1)-C(1) | -71.5(4) |
| N(3)-Co-O(1)-C(1) | 57(4) | N(4)-Co-O(1)-C(1) | -161.0(4) |
| N(5)-Co-O(1)-C(1) | 110.0(4) | O(1)-Co-N(1)-C(2) | -31.2(3) |
| N(2)-Co-N(1)-C(2) | 61.2(3) | N(3)-Co-N(1)-C(2) | 150.8(3) |
| N(4)-Co-N(1)-C(2) | -9(2) | N(5)-Co-N(1)-C(2) | -117.7(3) |
| O(1)-Co-N(2)-C(5) | 56.6(3) | N(1)-Co-N(2)-C(5) | -27.3(3) |
| N(3)-Co-N(2)-C(5) | -121.1(4) | N(4)-Co-N(2)-C(5) | 145.3(3) |
| N(5)-Co-N(2)-C(5) | 117(6) | Co-O(1)-C(1)-O(2) | -176.7(4) |
| Co-O(1)-C(1)-C(2) | 4.3(6) | Co-N(1)-C(2)-C(1) | 38.5(4) |
| Co-N(1)-C(2)-C(3) | 159.9(3) | Co-N(1)-C(2)-C(4) | -78.0(4) |
| Co-N(2)-C(5)-C(4) | .3(5) | Co-N(2)-C(5)-C(6) | 179.8(2) |
| O(1)-C(1)-C(2)-N(1) | -29.1(6) | O(1)-C(1)-C(2)-C(3) | -149.4(5) |
| O(1)-C(1)-C(2)-C(4) | 88.3(5) | O(2)-C(1)-C(2)-N(1) | 151.8(5) |
| O(2)-C(1)-C(2)-C(3) | 31.5(7) | O(2)-C(1)-C(2)-C(4) | -90.8(6) |
| N(1)-C(2)-C(4)-C(5) | 44.3(5) | C(1)-C(2)-C(4)-C(5) | -69.8(5) |
| C(3)-C(2)-C(4)-C(5) | 166.6(3) | C(2)-C(4)-C(5)-N(2) | -2.7(5) |
| C(2)-C(4)-C(5)-C(6) | 177.8(3) | N(2)-C(5)-C(6)-O(3) | 172.1(3) |
| N(2)-C(5)-C(6)-O(4) | -7.5(6) | C(4)-C(5)-C(6)-O(3) | -8.3(5) |
| C(4)-C(5)-C(6)-O(4) | 172.1(4) | | |

**Table SUP-IV. Additional distances (Å), angles (°) and torsion angles (°) for $[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2{\cdot}2H_2O$**

**a. the perchlorate anions**

**(Waser type restraints imposed during refinement: angles O-Cl-O= 109.5(20)° within A or B.)**

| | | | |
|---|---|---|---|
| Cl(1)-O(11A) | 1.33(1) | Cl(1)-O(12A) | 1.328(8) |
| Cl(1)-O(13A) | 1.442(9) | Cl(1)-O(14A) | 1.48(1) |
| Cl(1)-O(11B) | 1.39(2) | Cl(1)-O(12B) | 1.54(2) |
| Cl(1)-O(13B) | 1.32(2) | Cl(1)-O(14B) | 1.39(2) |
| Cl(2)-O(21A) | 1.37(1) | Cl(2)-O(22A) | 1.385(9) |
| Cl(2)-O(23A) | 1.38(1) | Cl(2)-O(24A) | 1.43(1) |
| Cl(2)-O(21B) | 1.48(2) | Cl(2)-O(22B) | 1.33(2) |
| Cl(2)-O(23B) | 1.36(2) | Cl(2)-O(24B) | 1.54(4) |
| | | | |
| O(11A)-Cl(1)-O(12A) | 120.4(9) | O(11A)-Cl(1)-O(13A) | 110.0(9) |
| O(11A)-Cl(1)-O(14A) | 107.2(8) | O(12A)-Cl(1)-O(13A) | 109.1(5) |
| O(12A)-Cl(1)-O(14A) | 106.9(7) | O(13A)-Cl(1)-O(14A) | 101.6(6) |
| O(11B)-Cl(1)-O(12B) | 103(1) | O(11B)-Cl(1)-O(13B) | 110(1) |
| O(11B)-Cl(1)-O(14B) | 106(1) | O(12B)-Cl(1)-O(13B) | 114.7(9) |
| O(12B)-Cl(1)-O(14B) | 104(1) | O(13B)-Cl(1)-O(14B) | 118(1) |
| O(21A)-Cl(2)-O(22A) | 112.7(7) | O(21A)-Cl(2)-O(23A) | 111.0(7) |
| O(21A)-Cl(2)-O(24A) | 109(1) | O(22A)-Cl(2)-O(23A) | 110.6(8) |
| O(22A)-Cl(2)-O(24A) | 106.6(8) | O(23A)-Cl(2)-O(24A) | 106.3(9) |
| O(21B)-Cl(2)-O(22B) | 113(1) | O(21B)-Cl(2)-O(23B) | 116(1) |
| O(21B)-Cl(2)-O(24B) | 99(2) | O(22B)-Cl(2)-O(23B) | 114(1) |
| O(22B)-Cl(2)-O(24B) | 108(1) | O(23B)-Cl(2)-O(24B) | 105(2) |

**b. involving hydrogen atoms**

| | | | |
|---|---|---|---|
| O(3)-H(1) | .795 | N(1)-H(2) | .927 |
| N(1)-H(3) | .956 | N(2)-H(4) | .801 |
| N(3)-H(10) | .850 | N(3)-H(11) | .850 |
| N(3)-H(12) | .850 | N(4)-H(13) | .850 |
| N(4)-H(14) | .850 | N(4)-H(15) | .850 |
| N(5)-H(16) | .850 | N(5)-H(17) | .850 |
| N(5)-H(18) | .850 | C(3)-H(5) | .921 |
| C(3)-H(6) | .923 | C(3)-H(7) | .918 |
| C(4)-H(8) | .940 | C(4)-H(9) | .920 |
| O(5)-H(19) | .666 | O(5)-H(20) | .979 |
| O(6)-H(21) | .806 | O(6)-H(22) | .966 |
| | | | |
| C(6)-O(3)-H(1) | 114.7 | Co-N(1)-H(2) | 115.3 |
| Co-N(1)-H(3) | 107.8 | C(2)-N(1)-H(2) | 111.2 |
| C(2)-N(1)-H(3) | 109.1 | H(2)-N(1)-H(3) | 106.9 |
| Co-N(2)-H(4) | 116.9 | C(5)-N(2)-H(4) | 113.5 |
| Co-N(3)-H(10) | 109.4 | Co-N(3)-H(11) | 109.4 |
| Co-N(3)-H(12) | 109.5 | H(10)-N(3)-H(11) | 109.5 |
| H(10)-N(3)-H(12) | 109.5 | H(11)-N(3)-H(12) | 109.5 |
| Co-N(4)-H(13) | 109.5 | Co-N(4)-H(14) | 109.5 |
| Co-N(4)-H(15) | 109.5 | H(13)-N(4)-H(14) | 109.5 |
| H(13)-N(4)-H(15) | 109.5 | H(14)-N(4)-H(15) | 109.5 |
| Co-N(5)-H(16) | 109.5 | Co-N(5)-H(17) | 109.5 |
| Co-N(5)-H(18) | 109.5 | H(16)-N(5)-H(17) | 109.5 |
| H(16)-N(5)-H(18) | 109.5 | H(17)-N(5)-H(18) | 109.5 |
| C(2)-C(3)-H(5) | 112.8 | C(2)-C(3)-H(6) | 110.5 |
| C(2)-C(3)-H(7) | 104.4 | H(5)-C(3)-H(6) | 110.6 |
| H(5)-C(3)-H(7) | 114.6 | H(6)-C(3)-H(7) | 103.3 |
| C(2)-C(4)-H(8) | 110.1 | C(2)-C(4)-H(9) | 104.1 |
| C(5)-C(4)-H(8) | 107.8 | C(5)-C(4)-H(9) | 107.7 |
| H(8)-C(4)-H(9) | 110.3 | H(19)-O(5)-H(20) | 120.8 |
| H(21)-O(6)-H(22) | 120.7 | | |
| | | | |
| O(1)-Co-N(1)-H(2) | -154.9 | O(1)-Co-N(1)-H(3) | 85.7 |
| N(2)-Co-N(1)-H(2) | -62.5 | N(2)-Co-N(1)-H(3) | 178.2 |
| N(3)-Co-N(1)-H(2) | 27.0 | N(3)-Co-N(1)-H(3) | -92.3 |
| N(4)-Co-N(1)-H(2) | -133 | N(4)-Co-N(1)-H(3) | 108 |
| N(5)-Co-N(1)-H(2) | 118.5 | N(5)-Co-N(1)-H(3) | -.8 |

| | | | |
|---|---|---|---|
| O(1)-Co-N(2)-H(4) | -120.9 | N(1)-Co-N(2)-H(4) | 155.3 |
| N(3)-Co-N(2)-H(4) | 61.4 | N(4)-Co-N(2)-H(4) | -32.2 |
| N(5)-Co-N(2)-H(4) | -60 | O(1)-Co-N(3)-H(10) | -110 |
| O(1)-Co-N(3)-H(11) | 9 | O(1)-Co-N(3)-H(12) | 130 |
| N(1)-Co-N(3)-H(10) | -69.9 | N(1)-Co-N(3)-H(11) | 50.0 |
| N(1)-Co-N(3)-H(12) | 170.1 | N(2)-Co-N(3)-H(10) | 17.8 |
| N(2)-Co-N(3)-H(11) | 137.8 | N(2)-Co-N(3)-H(12) | -102.2 |
| N(4)-Co-N(3)-H(10) | 107.3 | N(4)-Co-N(3)-H(11) | -132.7 |
| N(4)-Co-N(3)-H(12) | -12.7 | N(5)-Co-N(3)-H(10) | -163.7 |
| N(5)-Co-N(3)-H(11) | -43.7 | N(5)-Co-N(3)-H(12) | 76.3 |
| O(1)-Co-N(4)-H(13) | -69.4 | O(1)-Co-N(4)-H(14) | 50.6 |
| O(1)-Co-N(4)-H(15) | 170.6 | N(1)-Co-N(4)-H(13) | -92 |
| N(1)-Co-N(4)-H(14) | 28 | N(1)-Co-N(4)-H(15) | 148 |
| N(2)-Co-N(4)-H(13) | -161.6 | N(2)-Co-N(4)-H(14) | -41.6 |
| N(2)-Co-N(4)-H(15) | 78.4 | N(3)-Co-N(4)-H(13) | 108.7 |
| N(3)-Co-N(4)-H(14) | -131.2 | N(3)-Co-N(4)-H(15) | -11.2 |
| N(5)-Co-N(4)-H(13) | 17.6 | N(5)-Co-N(4)-H(14) | 137.6 |
| N(5)-Co-N(4)-H(15) | -102.4 | O(1)-Co-N(5)-H(16) | 163.5 |
| O(1)-Co-N(5)-H(17) | -76.5 | O(1)-Co-N(5)-H(18) | 43.5 |
| N(1)-Co-N(5)-H(16) | -112.8 | N(1)-Co-N(5)-H(17) | 7.2 |
| N(1)-Co-N(5)-H(18) | 127.2 | N(2)-Co-N(5)-H(16) | 103 |
| N(2)-Co-N(5)-H(17) | -137 | N(2)-Co-N(5)-H(18) | -17 |
| N(3)-Co-N(5)-H(16) | -18.9 | N(3)-Co-N(5)-H(17) | 101.1 |
| N(3)-Co-N(5)-H(18) | -138.9 | N(4)-Co-N(5)-H(16) | 74.7 |
| N(4)-Co-N(5)-H(17) | -165.3 | N(4)-Co-N(5)-H(18) | -45.3 |
| H(1)-O(3)-C(6)-O(4) | 3.7 | H(1)-O(3)-C(6)-C(5) | -175.9 |
| H(2)-N(1)-C(2)-C(1) | 164.8 | H(2)-N(1)-C(2)-C(3) | -73.8 |
| H(2)-N(1)-C(2)-C(4) | 48.2 | H(3)-N(1)-C(2)-C(1) | -77.5 |
| H(3)-N(1)-C(2)-C(3) | 43.9 | H(3)-N(1)-C(2)-C(4) | 165.9 |
| H(4)-N(2)-C(5)-C(4) | 177.8 | H(4)-N(2)-C(5)-C(6) | -2.6 |
| N(1)-C(2)-C(3)-H(5) | 179.9 | N(1)-C(2)-C(3)-H(6) | -55.7 |
| N(1)-C(2)-C(3)-H(7) | 54.8 | C(1)-C(2)-C(3)-H(5) | -63.3 |
| C(1)-C(2)-C(3)-H(6) | 61.1 | C(1)-C(2)-C(3)-H(7) | 171.6 |
| C(4)-C(2)-C(3)-H(5) | 58.1 | C(4)-C(2)-C(3)-H(6) | -177.5 |
| C(4)-C(2)-C(3)-H(7) | -67.0 | N(1)-C(2)-C(4)-H(8) | 167.5 |
| N(1)-C(2)-C(4)-H(9) | -74.2 | C(1)-C(2)-C(4)-H(8) | 53.4 |
| C(1)-C(2)-C(4)-H(9) | 171.7 | C(3)-C(2)-C(4)-H(8) | -70.1 |
| C(3)-C(2)-C(4)-H(9) | 48.1 | H(8)-C(4)-C(5)-N(2) | -127.1 |
| H(8)-C(4)-C(5)-C(6) | 53.4 | H(9)-C(4)-C(5)-N(2) | 113.9 |
| H(9)-C(4)-C(5)-C(6) | -65.6 | | |

**c. Intermolecular contacts of less than 2.6Å between hydrogen atoms and non-hydrogen atoms.**

| *X–H···Y(symmetry operation on Y)* | *X–H** | *H···Y* | *X·····Y* |
|---|---|---|---|
| O(3)–H(1)···O(5)(x,y+1,z) | 0.80 | 1.76 | 2.554(7) |
| N(1)–H(2)···O(24A)(x,y,z) | 0.93 | 2.27 | 3.14(1) |
| N(1)–H(2)···O(22B)(x,y,z) | | 2.47 | 3.25(2) |
| N(1)–H(3)···O(6)(x,y,z) | 0.96 | 2.05 | 2.962(7) |
| N(2)–H(4)···O(11B)(1-x,1-y,-z) | 0.80 | 2.31 | 2.95(2) |
| N(2)–H(4)···O(12A)(1-x,1-y,-z) | | 2.41 | 3.20(1) |
| N(3)–H(10)···O(24A)(x,y,z) | 0.85 | 2.42 | 3.02(1) |
| N(3)–H(10)···O(11B)(1-x,1-y,-z) | | 2.49 | 2.81(2) |
| N(3)–H(11)···O(24B)(2-x,1-y,1-z) | 0.85 | 2.16 | 2.95(4) |
| N(3)–H(11)···O(21A)(2-x,1-y,1-z) | | 2.37 | 3.16(2) |
| N(3)–H(12)···O(13B)(x+1,y,z) | 0.85 | 2.36 | 3.00(2) |
| N(3)–H(12)···O(14A)(x+1,y,z) | | 2.40 | 3.22(1) |
| N(3)–H(12)···O(11B)(1-x,1-y,-z) | | 2.49 | 2.81(2) |
| N(3)–H(12)···O(14A)(1-x,1-y,-z) | | 2.52 | 3.20(1) |
| N(4)–H(13)···O(2)(1-x,-y,-z) | 0.85 | 2.20 | 3.027(6) |
| N(4)–H(14)···O(12B)(x,y,z) | 0.85 | 2.40 | 3.17(2) |
| N(4)–H(14)···O(4)(1-x,1-y,-z) | | 2.53 | 3.067(6) |
| N(4)–H(15)···O(14A)(1-x,1-y,-z) | 0.85 | 2.32 | 3.16(1) |
| N(4)–H(15)···O(11B)(1-x,1-y,-z) | | 2.54 | 3.17(2) |
| N(5)–H(16)···O(13A)(x+1,y,z) | 0.85 | 2.44 | 3.28(1) |
| N(5)–H(16)···O(14B)(x+1,y,z) | | 2.52 | 3.15(3) |
| N(5)–H(17)···O(6)(x,y,z) | 0.85 | 2.10 | 2.934(8) |
| N(5)–H(18)···O(12B)(1-x,-y,-z) | 0.85 | 2.38 | 2.97(2) |
| O(5)–H(19)···O(23B)(x-1,y-1,z) | 0.67 | 2.27 | 2.78(2) |
| O(5)–H(19)···O(23A)(x-1,y-1,z) | | 2.32 | 2.96(1) |
| O(5)–H(20)···O(2)(x,y,z) | 0.98 | 1.84 | 2.816(7) |
| O(6)–H(21)···O(21B)(x,y-1,z) | 0.81 | 2.14 | 2.89(2) |
| O(6)–H(21)···O(23A)(x,y-1,z) | | 2.59 | 3.22(1) |
| O(6)–H(22)···O(4)(x,y-1,z) | 0.97 | 2.08 | 2.979(5) |

* Hydrogen atom positions come directly from a difference-Fourier map, except for those on N(3), N(4) and N(5) which are based on observed peaks but were adjusted so as to give tetrahedral angles at the nitrogen atom and N-H distances of 0.85Å.

**Table SUP-V. Selected least-squares planes for $[Co(NH_3)_3(C_6H_9N_2O_4)](ClO_4)_2{\cdot}2H_2O$.**

Atoms are weighted according to their estimated standard deviations. Deviations from the plane are given (in Å) for the atoms defining the plane and then for other atoms.

PLANE NUMBER 1

| <CHI**2> | <GOODNESS OF FIT> | <N-3> |
|---|---|---|
| 228.907 | 10.6983 | 2 |

| ATOM | | DIST(A) | ESDD |
|---|---|---|---|
| Co | DEFINING | -.0009 | .0010 |
| O1 | DEFINING | -.0253 | .0045 |
| N1 | DEFINING | .0441 | .0054 |
| N3 | DEFINING | -.0401 | .0058 |
| N4 | DEFINING | .0519 | .0058 |
| N2 | NON-DEFINING | 1.9175 | .0039 |
| N5 | NON-DEFINING | -1.9705 | .0055 |
| C1 | NON-DEFINING | .3139 | .0063 |
| C2 | NON-DEFINING | .7798 | .0064 |

PLANE NUMBER 2

| <CHI**2> | <GOODNESS OF FIT> | <N-3> |
|---|---|---|
| 976.956 | 22.1015 | 2 |

| ATOM | | DIST(A) | ESDD |
|---|---|---|---|
| Co | DEFINING | .0113 | .0010 |
| N1 | DEFINING | -.1037 | .0054 |
| N2 | DEFINING | -.0006 | .0050 |
| N4 | DEFINING | -.1262 | .0058 |
| N5 | DEFINING | -.0088 | .0065 |
| O1 | NON-DEFINING | -1.8754 | .0035 |
| N3 | NON-DEFINING | 1.9753 | .0049 |
| C2 | NON-DEFINING | -1.3907 | .0062 |
| C5 | NON-DEFINING | -.5058 | .0066 |

PLANE NUMBER 3

| <CHI**2> | <GOODNESS OF FIT> | <N-3> |
|---|---|---|
| 159.218 | 8.9224 | 2 |

| ATOM | | DIST(A) | ESDD |
|---|---|---|---|
| Co | DEFINING | -.0018 | .0010 |
| O1 | DEFINING | .0267 | .0045 |
| N2 | DEFINING | -.0183 | .0050 |
| N3 | DEFINING | .0492 | .0058 |
| N5 | DEFINING | -.0377 | .0065 |
| N1 | NON-DEFINING | 1.9313 | .0043 |
| N4 | NON-DEFINING | -1.9598 | .0048 |
| C1 | NON-DEFINING | 1.1289 | .0061 |
| C5 | NON-DEFINING | .8018 | .0065 |

**PLANE NUMBER 4**

| <CHI**2> | <GOODNESS OF FIT> | <N-3> |
|---|---|---|
| 1.2972 | 1.1389 | 1 |

| ATOM | | DIST(A) | ESDD |
|---|---|---|---|
| O1 | DEFINING | .0009 | .0045 |
| O2 | DEFINING | .0012 | .0048 |
| C1 | DEFINING | -.0053 | .0049 |
| C2 | DEFINING | .0015 | .0065 |
| Co | NON-DEFINING | -.1109 | .0063 |
| N1 | NON-DEFINING | -.6926 | .0074 |
| C3 | NON-DEFINING | -.7173 | .0089 |
| C4 | NON-DEFINING | 1.4505 | .0076 |

**PLANE NUMBER 5**

| <CHI**2> | <GOODNESS OF FIT> | <N-3> |
|---|---|---|
| .3436 | .5861 | 1 |

| ATOM | | DIST(A) | ESDD |
|---|---|---|---|
| N2 | DEFINING | .0006 | .0049 |
| C4 | DEFINING | .0010 | .0069 |
| C5 | DEFINING | -.0025 | .0047 |
| C6 | DEFINING | .0008 | .0065 |
| Co | NON-DEFINING | .0030 | .0075 |
| O3 | NON-DEFINING | -.1671 | .0076 |
| O4 | NON-DEFINING | .1400 | .0077 |
| C2 | NON-DEFINING | -.0550 | .0088 |
| H4 | NON-DEFINING | -.0295 | .0044 |

**PLANE NUMBER 6**

| <CHI**2> | <GOODNESS OF FIT> | <N-3> |
|---|---|---|
| .2423 | .4922 | 1 |

| ATOM | | DIST(A) | ESDD |
|---|---|---|---|
| O3 | DEFINING | -.0005 | .0051 |
| O4 | DEFINING | -.0007 | .0054 |
| C5 | DEFINING | -.0006 | .0062 |
| C6 | DEFINING | .0023 | .0050 |
| N2 | NON-DEFINING | -.1539 | .0074 |
| C4 | NON-DEFINING | .1816 | .0086 |
| H1 | NON-DEFINING | -.0509 | .0046 |

**PLANE NUMBER 7**

| <CHI**2> | <GOODNESS OF FIT> | <N-3> |
|---|---|---|
| 934.503 | 17.6494 | 3 |

| ATOM | | DIST(A) | ESDD |
|---|---|---|---|
| N2 | DEFINING | -.0664 | .0048 |
| O3 | DEFINING | -.0736 | .0048 |
| O4 | DEFINING | .0761 | .0051 |
| C4 | DEFINING | .1064 | .0064 |
| C5 | DEFINING | .0099 | .0048 |
| C6 | DEFINING | .0107 | .0051 |
| Co | NON-DEFINING | -.0904 | .0054 |
| C2 | NON-DEFINING | .0474 | .0073 |
| H1 | NON-DEFINING | -.1265 | .0037 |
| H4 | NON-DEFINING | -.1412 | .0034 |

**ANGLES BETWEEN PLANES (°)**

| Pln1 | Pln2 | Angle | Esd |
|---|---|---|---|
| 1 | 2 | 91.823 | .128 |
| 1 | 3 | 91.506 | .134 |
| 2 | 3 | 88.067 | .128 |
| 1 | 4 | 19.766 | .172 |
| 2 | 4 | 96.711 | .170 |
| 3 | 4 | 110.739 | .170 |
| 1 | 5 | 91.825 | .178 |
| 2 | 5 | 30.516 | .181 |
| 3 | 5 | 57.556 | .177 |
| 4 | 5 | 105.711 | .214 |
| 1 | 6 | 90.120 | .176 |
| 2 | 6 | 38.317 | .177 |
| 3 | 6 | 49.840 | .173 |
| 4 | 6 | 105.826 | .212 |
| 5 | 6 | 7.938 | .211 |
| 1 | 7 | 91.041 | .123 |
| 2 | 7 | 34.526 | .143 |
| 3 | 7 | 53.573 | .138 |
| 4 | 7 | 105.899 | .171 |
| 5 | 7 | 4.071 | .183 |
| 6 | 7 | 3.869 | .179 |

# Appendix II: Data from the X-ray crystallographic analysis of $[Co(C_{11}H_{24}N_5O_2Br_2)]$

*EXPERIMENTAL DETAILS*

## A. Crystal Data

| | |
|---|---|
| Empirical Formula | $C_{11}H_{24}Br_2CoN_5O_2$ |
| Formula Weight | 477.08 |
| Crystal Colour, Habit | orange, irregular |
| Crystal Dimensions | 0.20 x 0.12 x 0.09 mm |
| Crystal System | monoclinic |
| Lattice Type | Primitive |
| No. of Reflections Used for Unit Cell Determination ($2\theta$ range) | 25 ( 35.3 - 46.0° ) |
| Omega Scan Peak Width at Half-height | 0.34° |
| Lattice Parameters | a = 7.790(3) Å<br>b = 23.598(2) Å<br>c = 9.652(3) Å<br>$\beta$ = 102.83(3)°<br><br>V = 1730.0(8) $Å^3$ |
| Space Group | $P2_1/a$ (#14) |
| Z value | 4 |
| $D_{calc}$ | 1.832 $g/cm^3$ |
| $F_{000}$ | 952.00 |
| $\mu(MoK\alpha)$ | 56.36 $cm^{-1}$ |

B. Intensity Measurements

| | |
|---|---|
| Diffractometer | Rigaku AFC6S |
| Radiation | MoK$\alpha$ ($\lambda = 0.71069$ Å)<br>graphite monochromated |
| Take-off Angle | 6.0° |
| Detector Aperture | 3.5 mm horizontal<br>3.5 mm vertical |
| Crystal to Detector Distance | 200 mm |
| Temperature | 23.0°C |
| Scan Type | $\omega$-$2\theta$ |
| Scan Rate | 2.0°/min (in $\omega$) (up to 4 scans) |
| Scan Width | $(0.90 + 0.34 \tan \theta)°$ |
| $2\theta_{max}$ | 50.1° |
| No. of Reflections Measured | Total: 3399<br>Unique: 3159 ($R_{int} = 0.026$) |
| Corrections | Lorentz-polarization<br>Absorption<br>(trans. factors: 0.8124 - 1.0000)<br>Secondary Extinction<br>(coefficient: 1.6(1)e-07) |

C. Structure Solution and Refinement

| | |
|---|---|
| Structure Solution | Patterson Methods (DIRDIF92 PATTY) |
| Refinement | Full-matrix least-squares |
| Function Minimized | $\Sigma w(\lvert Fo\rvert - \lvert Fc\rvert)^2$ |
| Least Squares Weights | $\frac{1}{\sigma^2(Fo)} = \frac{4Fo^2}{\sigma^2(Fo^2)}$ |
| p-factor | 0.001 |
| Anomalous Dispersion | All non-hydrogen atoms |
| No. Observations (I>3.00$\sigma$(I)) | 1919 |
| No. Variables | 201 |
| Reflection/Parameter Ratio | 9.55 |
| Residuals: R; Rw | 0.031 ; 0.021 |
| Goodness of Fit Indicator | 1.59 |
| Max Shift/Error in Final Cycle | 0.09 |
| Maximum peak in Final Diff. Map | 0.44 $e^-/\mathring{A}^3$ |
| Minimum peak in Final Diff. Map | -0.39 $e^-/\mathring{A}^3$ |

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| Br(1) | -0.17207(8) | -0.06099(2) | -0.36018(6) | 3.69(1) |
| Br(2) | 0.20772(7) | -0.28683(2) | 0.10144(7) | 4.08(1) |
| Co(1) | -0.31900(8) | -0.40249(3) | 0.10473(7) | 1.98(1) |
| O(1) | -0.1392(4) | -0.5502(1) | 0.0199(3) | 2.73(8) |
| O(2) | -0.2969(4) | -0.4706(1) | 0.0011(3) | 2.25(8) |
| N(1) | -0.4766(5) | -0.4415(2) | 0.2024(4) | 2.50(9) |
| N(2) | -0.3114(5) | -0.3429(2) | 0.2480(4) | 2.68(10) |
| N(3) | -0.1178(4) | -0.4309(1) | 0.2300(4) | 1.86(9) |
| N(4) | -0.1760(5) | -0.3626(2) | -0.0085(4) | 3.0(1) |
| N(5) | -0.5208(5) | -0.3745(2) | -0.0382(4) | 2.60(10) |
| C(1)* | -0.562(1) | -0.4054(4) | 0.2836(10) | 2.5(2) |
| C(1')** | -0.467(2) | -0.4101(7) | 0.344(2) | 3.9(4) |
| C(2) | -0.4277(7) | -0.3580(3) | 0.3456(6) | 4.2(2) |
| C(3) | -0.1195(7) | -0.3362(2) | 0.3247(5) | 3.2(1) |
| C(4) | -0.0961(7) | -0.2978(2) | 0.4511(6) | 4.1(1) |
| C(5) | 0.0073(8) | -0.3061(3) | 0.5745(6) | 5.7(2) |
| C(6) | -0.0419(6) | -0.3956(2) | 0.3534(5) | 3.2(1) |
| C(7) | -0.0626(6) | -0.4791(2) | 0.2022(5) | 2.2(1) |
| C(8) | 0.0893(6) | -0.5114(2) | 0.2868(5) | 3.0(1) |
| C(9) | -0.1715(6) | -0.5039(2) | 0.0641(5) | 2.2(1) |
| C(10) | -0.2860(7) | -0.3501(2) | -0.1516(5) | 3.3(1) |
| C(11) | -0.4626(7) | -0.3317(2) | -0.1313(5) | 3.5(1) |
| H(01a)* | -0.5643 | -0.4602 | 0.1335 | 2.9959 |
| H(01'a)** | -0.5936 | -0.4405 | 0.1465 | 2.9957 |

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| H(01b)* | -0.4101 | -0.4687 | 0.2646 | 2.9959 |
| H(01'b)** | -0.4403 | -0.4797 | 0.2200 | 2.9957 |
| H(1a)* | -0.6645 | -0.3893 | 0.2248 | 2.9566 |
| H(1'a)** | -0.5797 | -0.4132 | 0.3674 | 4.7024 |
| H(1b)* | -0.5940 | -0.4261 | 0.3585 | 2.9566 |
| H(1'b)** | -0.3816 | -0.4286 | 0.4155 | 4.7024 |
| H(2a)* | -0.3565 | -0.3708 | 0.4330 | 4.9994 |
| H(2'a)** | -0.3691 | -0.3479 | 0.4396 | 4.9994 |
| H(2b) | -0.4910 | -0.3251 | 0.3620 | 4.9994 |
| H(2'b) | -0.5336 | -0.3368 | 0.3187 | 4.9994 |
| H(02) | -0.3514 | -0.3084 | 0.2012 | 3.2200 |
| H(3) | -0.0608 | -0.3187 | 0.2595 | 3.8672 |
| H(4) | -0.1641 | -0.2640 | 0.4397 | 4.9283 |
| H(04a) | -0.1333 | -0.3281 | 0.0374 | 3.5388 |
| H(04b) | -0.0794 | -0.3858 | -0.0176 | 3.5388 |
| H(05a) | -0.5749 | -0.4053 | -0.0947 | 3.1176 |
| H(05b) | -0.6032 | -0.3575 | 0.0083 | 3.1176 |
| H(5a) | 0.0125 | -0.2789 | 0.6481 | 6.8185 |
| H(5b) | 0.0781 | -0.3392 | 0.5914 | 6.8185 |
| H(6a) | -0.0711 | -0.4109 | 0.4363 | 3.8184 |
| H(6b) | 0.0825 | -0.3942 | 0.3660 | 3.8184 |
| H(8b) | 0.0518 | -0.5483 | 0.3057 | 3.5836 |
| H(8c) | 0.1790 | -0.5141 | 0.2346 | 3.5836 |
| H(8a) | 0.1341 | -0.4923 | 0.3739 | 3.5836 |

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{11}H_{24}Br_2CoN_5O_2$ (cont...)

| atom | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| H(10a) | -0.2338 | -0.3206 | -0.1954 | 4.0170 |
| H(10b) | -0.2978 | -0.3830 | -0.2094 | 4.0170 |
| H(11a) | -0.5442 | -0.3300 | -0.2205 | 4.2481 |
| H(11b) | -0.4540 | -0.2954 | -0.0875 | 4.2481 |

$$B_{eq} = \frac{8}{3}\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha)$$

populations: *0.59(1) **0.41(1)

Table 2. Anisotropic Displacement Parameters for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | $U_{11}$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{13}$ | $U_{23}$ |
|---|---|---|---|---|---|---|
| Br(1) | 0.0604(4) | 0.0451(3) | 0.0313(3) | 0.0024(3) | 0.0028(3) | -0.0029(3) |
| Br(2) | 0.0418(3) | 0.0367(3) | 0.0781(5) | 0.0044(3) | 0.0169(3) | -0.0043(3) |
| Co(1) | 0.0258(3) | 0.0266(4) | 0.0230(4) | 0.0001(3) | 0.0056(3) | -0.0002(3) |
| O(1) | 0.044(2) | 0.026(2) | 0.035(2) | 0.002(2) | 0.012(2) | -0.006(2) |
| O(2) | 0.028(2) | 0.031(2) | 0.025(2) | 0.003(2) | 0.001(2) | -0.004(2) |
| N(1) | 0.025(2) | 0.042(3) | 0.028(2) | 0.000(2) | 0.004(2) | 0.001(2) |
| N(2) | 0.043(3) | 0.028(2) | 0.029(3) | 0.005(2) | 0.003(2) | 0.003(2) |
| N(3) | 0.024(2) | 0.024(2) | 0.024(2) | -0.007(2) | 0.007(2) | -0.005(2) |
| N(4) | 0.040(3) | 0.035(3) | 0.037(3) | -0.005(2) | 0.010(2) | -0.001(2) |
| N(5) | 0.035(2) | 0.034(2) | 0.030(3) | 0.005(2) | 0.009(2) | -0.003(2) |
| C(1) | 0.028(5) | 0.028(5) | 0.040(6) | -0.002(5) | 0.013(4) | 0.012(5) |
| C(1') | 0.06(1) | 0.06(1) | 0.043(10) | -0.002(9) | 0.035(7) | -0.002(9) |
| C(2) | 0.039(3) | 0.079(5) | 0.043(4) | 0.003(3) | 0.016(3) | -0.014(4) |
| C(3) | 0.056(4) | 0.032(3) | 0.035(3) | -0.003(3) | 0.011(3) | -0.003(3) |
| C(4) | 0.074(4) | 0.036(4) | 0.043(4) | -0.006(3) | 0.008(3) | -0.016(3) |
| C(5) | 0.088(5) | 0.067(4) | 0.052(4) | 0.004(4) | -0.003(4) | -0.030(4) |
| C(6) | 0.040(3) | 0.043(3) | 0.036(3) | 0.001(3) | 0.005(3) | -0.009(3) |
| C(7) | 0.027(3) | 0.031(3) | 0.025(3) | -0.001(2) | 0.006(2) | 0.002(2) |
| C(8) | 0.036(3) | 0.038(3) | 0.036(3) | 0.005(3) | 0.003(3) | -0.005(3) |
| C(9) | 0.026(3) | 0.033(3) | 0.028(3) | -0.005(2) | 0.015(2) | 0.002(2) |
| C(10) | 0.052(4) | 0.045(3) | 0.031(3) | -0.007(3) | 0.011(3) | 0.009(3) |
| C(11) | 0.062(4) | 0.033(3) | 0.035(3) | 0.003(3) | 0.002(3) | 0.004(3) |

The general temperature factor expression:

$$\exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$$

Table 3. Interatomic Distances (Å) Involving Non-Hydrogen Atoms for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | atom | distance | atom | atom | distance |
|---|---|---|---|---|---|
| Co(1) | O(2) | 1.921(3) | Co(1) | N(1) | 1.938(4) |
| Co(1) | N(2) | 1.964(4) | Co(1) | N(3) | 1.877(3) |
| Co(1) | N(4) | 1.965(4) | Co(1) | N(5) | 1.962(4) |
| O(1) | C(9) | 1.219(5) | O(2) | C(9) | 1.295(5) |
| N(1) | C(1) | 1.419(10) | N(1) | C(1') | 1.55(1) |
| N(2) | C(2) | 1.487(6) | N(2) | C(3) | 1.522(6) |
| N(3) | C(6) | 1.465(5) | N(3) | C(7) | 1.266(5) |
| N(4) | C(10) | 1.484(6) | N(5) | C(11) | 1.487(6) |
| C(1) | C(1') | 0.84(1) | C(1) | C(2) | 1.56(1) |
| C(1') | C(2) | 1.27(2) | C(3) | C(4) | 1.497(6) |
| C(3) | C(6) | 1.528(6) | C(4) | C(5) | 1.296(7) |
| C(7) | C(8) | 1.490(6) | C(7) | C(9) | 1.529(6) |
| C(10) | C(11) | 1.496(7) | | | |

Table 4. Interatomic Distances (Å) Involving Hydrogen Atoms for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | atom | distance | atom | atom | distance |
|---|---|---|---|---|---|
| N(1) | H(01a) | 0.95 | N(1) | H(01'a) | 0.95 |
| N(1) | H(01b) | 0.95 | N(1) | H(01'b) | 0.95 |
| N(2) | H(02) | 0.95 | N(4) | H(04a) | 0.95 |
| N(4) | H(04b) | 0.95 | N(5) | H(05a) | 0.95 |
| N(5) | H(05b) | 0.95 | C(1) | H(1a) | 0.95 |
| C(1) | H(1b) | 0.95 | C(1') | H(1'a) | 0.95 |
| C(1') | H(1'b) | 0.95 | C(2) | H(2a) | 0.95 |
| C(2) | H(2'a) | 0.95 | C(2) | H(2b) | 0.95 |
| C(2) | H(2'b) | 0.95 | C(3) | H(3) | 0.95 |
| C(4) | H(4) | 0.95 | C(5) | H(5a) | 0.95 |
| C(5) | H(5b) | 0.95 | C(6) | H(6a) | 0.95 |
| C(6) | H(6b) | 0.95 | C(8) | H(8b) | 0.95 |
| C(8) | H(8c) | 0.95 | C(8) | H(8a) | 0.95 |
| C(10) | H(10a) | 0.95 | C(10) | H(10b) | 0.95 |
| C(11) | H(11a) | 0.95 | C(11) | H(11b) | 0.95 |

Table 5. Interatomic Angles (°) Involving Non-Hydrogen Atoms for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | atom | atom | angle | atom | atom | atom | angle |
|---|---|---|---|---|---|---|---|
| O(2) | Co(1) | N(1) | 89.8(1) | O(2) | Co(1) | N(2) | 166.8(1) |
| O(2) | Co(1) | N(3) | 83.0(1) | O(2) | Co(1) | N(4) | 89.1(1) |
| O(2) | Co(1) | N(5) | 93.8(1) | N(1) | Co(1) | N(2) | 85.4(2) |
| N(1) | Co(1) | N(3) | 92.6(1) | N(1) | Co(1) | N(4) | 175.1(2) |
| N(1) | Co(1) | N(5) | 90.4(2) | N(2) | Co(1) | N(3) | 84.9(1) |
| N(2) | Co(1) | N(4) | 96.7(2) | N(2) | Co(1) | N(5) | 98.5(2) |
| N(3) | Co(1) | N(4) | 91.9(2) | N(3) | Co(1) | N(5) | 175.6(2) |
| N(4) | Co(1) | N(5) | 84.9(2) | Co(1) | O(2) | C(9) | 114.1(3) |
| Co(1) | N(1) | C(1) | 114.2(4) | Co(1) | N(1) | C(1') | 107.2(6) |
| Co(1) | N(2) | C(2) | 110.3(3) | Co(1) | N(2) | C(3) | 106.9(3) |
| C(2) | N(2) | C(3) | 112.8(4) | Co(1) | N(3) | C(6) | 116.7(3) |
| Co(1) | N(3) | C(7) | 117.3(3) | C(6) | N(3) | C(7) | 126.0(4) |
| Co(1) | N(4) | C(10) | 109.4(3) | Co(1) | N(5) | C(11) | 110.5(3) |
| N(1) | C(1) | C(2) | 106.6(6) | N(1) | C(1') | C(2) | 115(1) |
| N(2) | C(2) | C(1) | 112.3(5) | N(2) | C(2) | C(1') | 114.1(8) |
| N(2) | C(3) | C(4) | 112.8(4) | N(2) | C(3) | C(6) | 107.3(4) |
| C(4) | C(3) | C(6) | 115.8(4) | C(3) | C(4) | C(5) | 126.6(5) |
| N(3) | C(6) | C(3) | 107.6(4) | N(3) | C(7) | C(8) | 127.6(4) |
| N(3) | C(7) | C(9) | 112.3(4) | C(8) | C(7) | C(9) | 120.1(4) |
| O(1) | C(9) | O(2) | 124.9(4) | O(1) | C(9) | C(7) | 122.0(4) |
| O(2) | C(9) | C(7) | 113.1(4) | N(4) | C(10) | C(11) | 107.1(4) |
| N(5) | C(11) | C(10) | 107.2(4) | | | | |

Table 6. Interatomic Angles (°) Involving Hydrogen Atoms for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | atom | atom | angle | atom | atom | atom | angle |
|---|---|---|---|---|---|---|---|
| Co(1) | N(1) | H(01a) | 108.2 | Co(1) | N(1) | H(01'a) | 110.1 |
| Co(1) | N(1) | H(01b) | 108.2 | Co(1) | N(1) | H(01'b) | 110.0 |
| C(1) | N(1) | H(01a) | 108.4 | C(1) | N(1) | H(01b) | 108.3 |
| C(1') | N(1) | H(01'a) | 110.0 | C(1') | N(1) | H(01'b) | 110.1 |
| H(01a) | N(1) | H(01b) | 109.4 | H(01'a) | N(1) | H(01'b) | 109.5 |
| Co(1) | N(2) | H(02) | 108.9 | C(2) | N(2) | H(02) | 109.0 |
| C(3) | N(2) | H(02) | 108.9 | Co(1) | N(4) | H(04a) | 109.5 |
| Co(1) | N(4) | H(04b) | 109.5 | C(10) | N(4) | H(04a) | 109.5 |
| C(10) | N(4) | H(04b) | 109.5 | H(04a) | N(4) | H(04b) | 109.4 |
| Co(1) | N(5) | H(05a) | 109.2 | Co(1) | N(5) | H(05b) | 109.2 |
| C(11) | N(5) | H(05a) | 109.2 | C(11) | N(5) | H(05b) | 109.2 |
| H(05a) | N(5) | H(05b) | 109.5 | N(1) | C(1) | H(1a) | 109.9 |
| N(1) | C(1) | H(1b) | 110.5 | C(2) | C(1) | H(1a) | 110.2 |
| C(2) | C(1) | H(1'a) | 110.5 | C(2) | C(1) | H(1b) | 109.8 |
| H(1a) | C(1) | H(1b) | 109.4 | N(1) | C(1') | H(1'a) | 107.9 |
| N(1) | C(1') | H(1'b) | 107.8 | C(2) | C(1') | H(1'a) | 107.9 |
| C(2) | C(1') | H(1'b) | 107.8 | H(1'a) | C(1') | H(1'b) | 105.2 |
| N(2) | C(2) | H(2a) | 108.7 | N(2) | C(2) | H(2'a) | 108.3 |
| N(2) | C(2) | H(2b) | 108.7 | N(2) | C(2) | H(2'b) | 108.4 |
| C(1) | C(2) | H(2a) | 109.0 | C(1) | C(2) | H(2b) | 108.7 |
| C(1') | C(2) | H(2'a) | 108.3 | C(1') | C(2) | H(2'b) | 108.2 |
| H(2a) | C(2) | H(2b) | 109.4 | H(2'a) | C(2) | H(2'b) | 109.5 |
| N(2) | C(3) | H(3) | 106.8 | C(4) | C(3) | H(3) | 106.8 |
| C(6) | C(3) | H(3) | 106.8 | C(3) | C(4) | H(4) | 116.7 |

Table 6. Interatomic Angles (°) Involving Hydrogen Atoms for $C_{11}H_{24}Br_2CoN_5O_2$ (cont...)

| atom | atom | atom | angle | atom | atom | atom | angle |
|---|---|---|---|---|---|---|---|
| C(5) | C(4) | H(4) | 116.7 | C(4) | C(5) | H(5a) | 120.0 |
| C(4) | C(5) | H(5b) | 120.0 | H(5a) | C(5) | H(5b) | 120.0 |
| N(3) | C(6) | H(6a) | 109.9 | N(3) | C(6) | H(6b) | 109.9 |
| C(3) | C(6) | H(6a) | 109.9 | C(3) | C(6) | H(6b) | 110.0 |
| H(6a) | C(6) | H(6b) | 109.5 | C(7) | C(8) | H(8b) | 109.5 |
| C(7) | C(8) | H(8c) | 109.5 | C(7) | C(8) | H(8a) | 109.5 |
| H(8b) | C(8) | H(8c) | 109.5 | H(8b) | C(8) | H(8a) | 109.5 |
| H(8c) | C(8) | H(8a) | 109.5 | N(4) | C(10) | H(10a) | 110.1 |
| N(4) | C(10) | H(10b) | 110.1 | C(11) | C(10) | H(10a) | 110.1 |
| C(11) | C(10) | H(10b) | 110.1 | H(10a) | C(10) | H(10b) | 109.4 |
| N(5) | C(11) | H(11a) | 110.1 | N(5) | C(11) | H(11b) | 110.1 |
| C(10) | C(11) | H(11a) | 110.1 | C(10) | C(11) | H(11b) | 110.1 |
| H(11a) | C(11) | H(11b) | 109.4 | | | | |

Table 7. Torsion Angles (°) Involving Non-Hydrogen atoms for $C_{11}H_{24}Br_2CoN_5O_2$

| atom | atom | atom | atom | angle | atom | atom | atom | atom | angle |
|---|---|---|---|---|---|---|---|---|---|
| Co(1) | O(2) | C(9) | O(1) | 176.9(4) | Co(1) | O(2) | C(9) | C(7) | -3.7(5) |
| Co(1) | N(1) | C(1) | C(1') | -84(1) | Co(1) | N(1) | C(1) | C(2) | -35.1(7) |
| Co(1) | N(1) | C(1') | C(1) | 108(1) | Co(1) | N(1) | C(1') | C(2) | 27(1) |
| Co(1) | N(2) | C(2) | C(1) | -17.0(6) | Co(1) | N(2) | C(2) | C(1') | 18.5(10) |
| Co(1) | N(2) | C(3) | C(4) | -171.9(3) | Co(1) | N(2) | C(3) | C(6) | -43.1(4) |
| Co(1) | N(3) | C(6) | C(3) | -15.7(5) | Co(1) | N(3) | C(7) | C(8) | -177.9(4) |
| Co(1) | N(3) | C(7) | C(9) | 2.4(5) | Co(1) | N(4) | C(10) | C(11) | 40.8(4) |
| Co(1) | N(5) | C(11) | C(10) | 35.9(5) | O(1) | C(9) | C(7) | N(3) | -179.6(4) |
| O(1) | C(9) | C(7) | C(8) | 0.6(7) | O(2) | Co(1) | N(1) | C(1) | -170.0(5) |
| O(2) | Co(1) | N(1) | C(1') | 155.9(7) | O(2) | Co(1) | N(2) | C(2) | -70.6(8) |
| O(2) | Co(1) | N(2) | C(3) | 52.4(8) | O(2) | Co(1) | N(3) | C(6) | 178.1(3) |
| O(2) | Co(1) | N(3) | C(7) | -3.5(3) | O(2) | Co(1) | N(4) | C(10) | 77.0(3) |
| O(2) | Co(1) | N(5) | C(11) | -99.6(3) | O(2) | C(9) | C(7) | N(3) | 1.0(6) |
| O(2) | C(9) | C(7) | C(8) | -178.8(4) | N(1) | Co(1) | O(2) | C(9) | -88.7(3) |
| N(1) | Co(1) | N(2) | C(2) | -1.6(3) | N(1) | Co(1) | N(2) | C(3) | 121.3(3) |
| N(1) | Co(1) | N(3) | C(6) | -92.5(3) | N(1) | Co(1) | N(3) | C(7) | 85.9(4) |
| N(1) | Co(1) | N(4) | C(10) | 0(2) | N(1) | Co(1) | N(5) | C(11) | 170.6(3) |
| N(1) | C(1) | C(1') | C(2) | 117.1(8) | N(1) | C(1) | C(2) | N(2) | 33.3(8) |
| N(1) | C(1) | C(2) | C(1') | -66(1) | N(1) | C(1') | C(1) | C(2) | -117.1(8) |
| N(1) | C(1') | C(2) | N(2) | -30(1) | N(1) | C(1') | C(2) | C(1) | 64(1) |
| N(2) | Co(1) | O(2) | C(9) | -20.2(8) | N(2) | Co(1) | N(1) | C(1) | 22.3(5) |
| N(2) | Co(1) | N(1) | C(1') | -11.8(7) | N(2) | Co(1) | N(3) | C(6) | -7.3(3) |
| N(2) | Co(1) | N(3) | C(7) | 171.1(4) | N(2) | Co(1) | N(4) | C(10) | -114.9(3) |
| N(2) | Co(1) | N(5) | C(11) | 85.2(3) | N(2) | C(2) | C(1) | C(1') | 100(1) |

Table 7. Torsion Angles (°) Involving Non-Hydrogen Atoms for $C_{11}H_{24}Br_2CoN_5O_2$ (cont...)

| atom | atom | atom | atom | angle | atom | atom | atom | atom | angle |
|---|---|---|---|---|---|---|---|---|---|
| N(2) | C(2) | C(1') | C(1) | -94(1) | N(2) | C(3) | C(4) | C(5) | 137.2(6) |
| N(2) | C(3) | C(6) | N(3) | 37.7(5) | N(3) | Co(1) | O(2) | C(9) | 4.0(3) |
| N(3) | Co(1) | N(1) | C(1) | 107.0(5) | N(3) | Co(1) | N(1) | C(1') | 72.8(7) |
| N(3) | Co(1) | N(2) | C(2) | -94.7(3) | N(3) | Co(1) | N(2) | C(3) | 28.3(3) |
| N(3) | Co(1) | N(4) | C(10) | 160.0(3) | N(3) | Co(1) | N(5) | C(11) | -55(2) |
| N(3) | C(6) | C(3) | C(4) | 164.8(4) | N(4) | Co(1) | O(2) | C(9) | 96.1(3) |
| N(4) | Co(1) | N(1) | C(1) | -93(1) | N(4) | Co(1) | N(1) | C(1') | -127(1) |
| N(4) | Co(1) | N(2) | C(2) | 173.9(3) | N(4) | Co(1) | N(2) | C(3) | -63.1(3) |
| N(4) | Co(1) | N(3) | C(6) | 89.3(3) | N(4) | Co(1) | N(3) | C(7) | -92.4(4) |
| N(4) | Co(1) | N(5) | C(11) | -10.8(3) | N(4) | C(10) | C(11) | N(5) | -49.5(5) |
| N(5) | Co(1) | O(2) | C(9) | -179.1(3) | N(5) | Co(1) | N(1) | C(1) | -76.2(5) |
| N(5) | Co(1) | N(1) | C(1') | -110.3(7) | N(5) | Co(1) | N(2) | C(2) | 88.1(3) |
| N(5) | Co(1) | N(2) | C(3) | -148.9(3) | N(5) | Co(1) | N(3) | C(6) | 133(1) |
| N(5) | Co(1) | N(3) | C(7) | -47(2) | N(5) | Co(1) | N(4) | C(10) | -16.9(3) |
| C(1) | N(1) | C(1') | C(2) | -80(1) | C(1) | C(2) | N(2) | C(3) | -136.5(5) |
| C(1') | N(1) | C(1) | C(2) | 49(1) | C(1') | C(2) | N(2) | C(3) | -100.9(9) |
| C(2) | N(2) | C(3) | C(4) | -50.4(6) | C(2) | N(2) | C(3) | C(6) | 78.4(5) |
| C(3) | C(6) | N(3) | C(7) | 166.0(4) | C(5) | C(4) | C(3) | C(6) | 12.9(9) |
| C(6) | N(3) | C(7) | C(8) | 0.4(8) | C(6) | N(3) | C(7) | C(9) | -179.4(4) |

# Appendix III: Notes on $^{31}P$ nmr chemical shifts

Phosphorus species are identified on application of a set of empirically derived 'additivity rules'.[1,2] Thus, the effect of the substituents on the chemical shifts are added onto the chemical shift of the 'parent' phosphate in order to derive the chemical shift of the molecule being characterised. Some of the substituent effects for addition to a basic oxygen of a phosphate are given in Table 1:

***Table 1: Substituent effects for addition to a basic oxygen of phosphate derivatives.***

| Substituent | $\Delta\delta$ |
|---|---|
| Co(III) | +6 to +8 |
| $H^+$ | -2 to -4 |
| $-CH_3$ | 0 to -2 |
| $-C_2H_5$ | 0 to -2 |
| $-C_6H_4NO_2$ | -5 to -6 |
| $-PO_3$ | -10 to -13 |

In addition to these, a special case exists on chelation of the substrate. In this instance an empirical correction of 4 to 5 ppm in addition to the componant for each bonded atom needs to be made (this effect is due to the change in O-P-O angle at the P centre).[5]

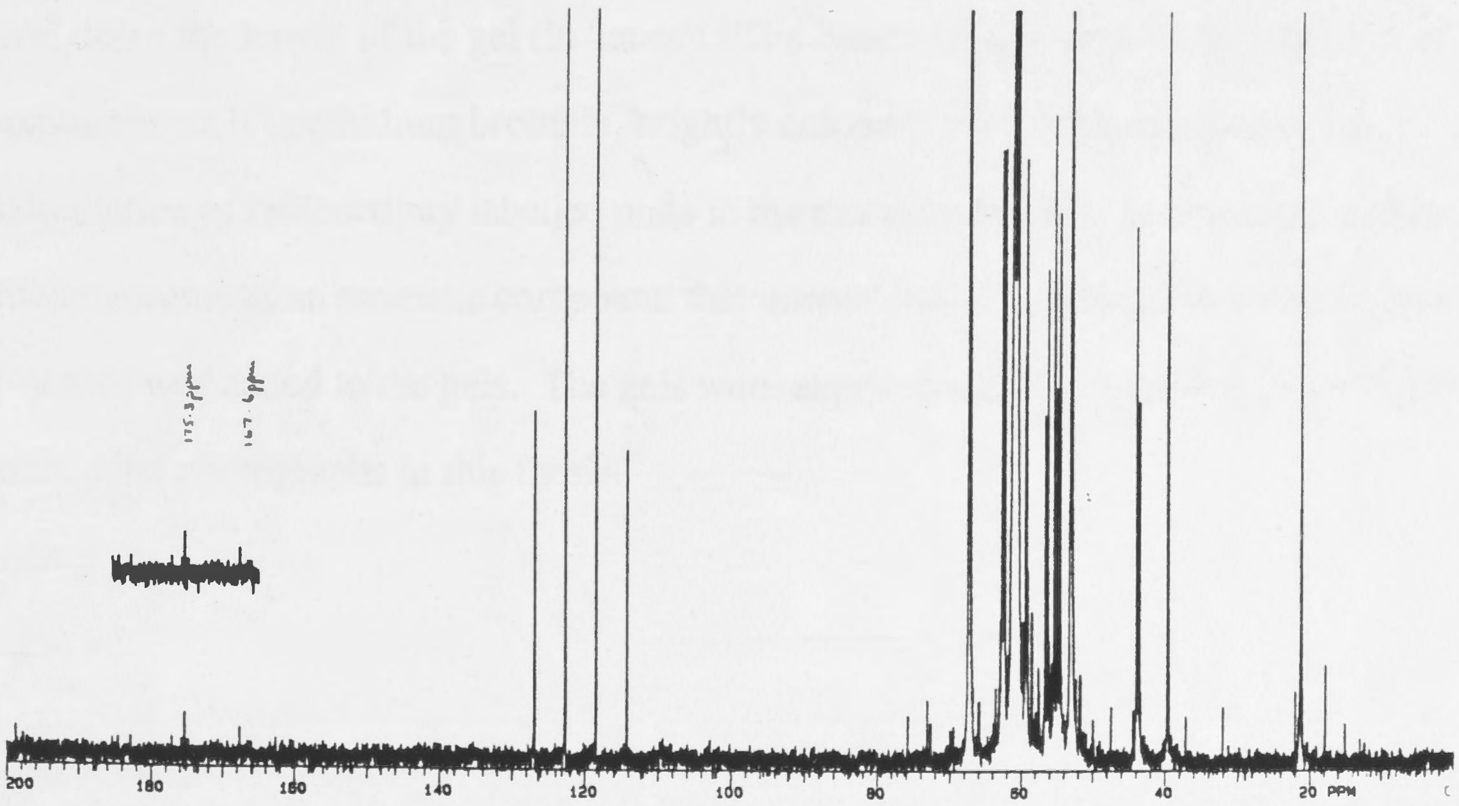
References

1 Hendry, P., Ph.D. Thesis, The Australian National University, 1985.

2 Wijesekera, R., Ph.D. Thesis, The Australian National University, 1990, and references therein.

## Appendix IV: Example of $^{13}C$ nmr spectra of reaction mixtures produced on the hydrolysis of coordinated carbamoyl phosphate by $[tamenCo(OH)(OH_2)]^{2+}$.

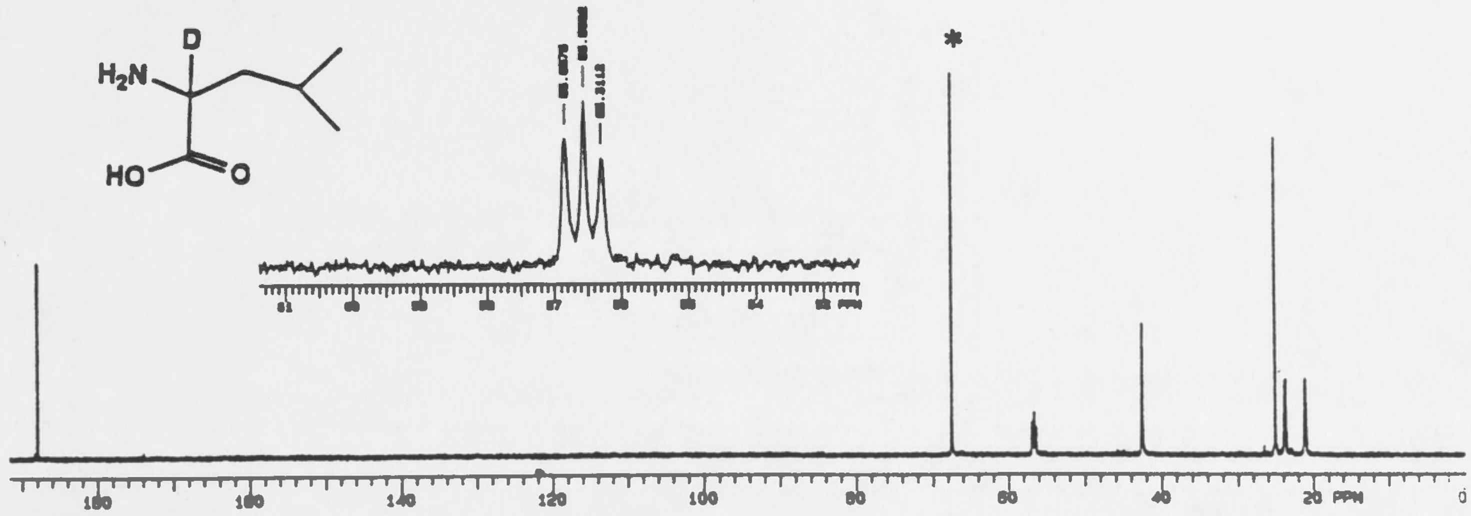
The signals due to the carbonyl-containing products appear in the region 160 - 180 ppm. Reaction conditions: $[(NH_3)_5Co(OPO_3CONH_2)]^+$ (7.5 x $10^{-5}$ moles) and $[tamenCo(CF_3SO_3)]CF_3SO_3$ (1.5 x $10^{-4}$ moles) were dissolved in Bis-Tris buffer (1 M, pH 7.5, 20% $D_2O$) and reacted for 30 minutes at 25 °C. A $^{13}C$ nmr spectrum, with dioxane as an internal standard, of this solution was acquired (below); this took ~ 1 hour.

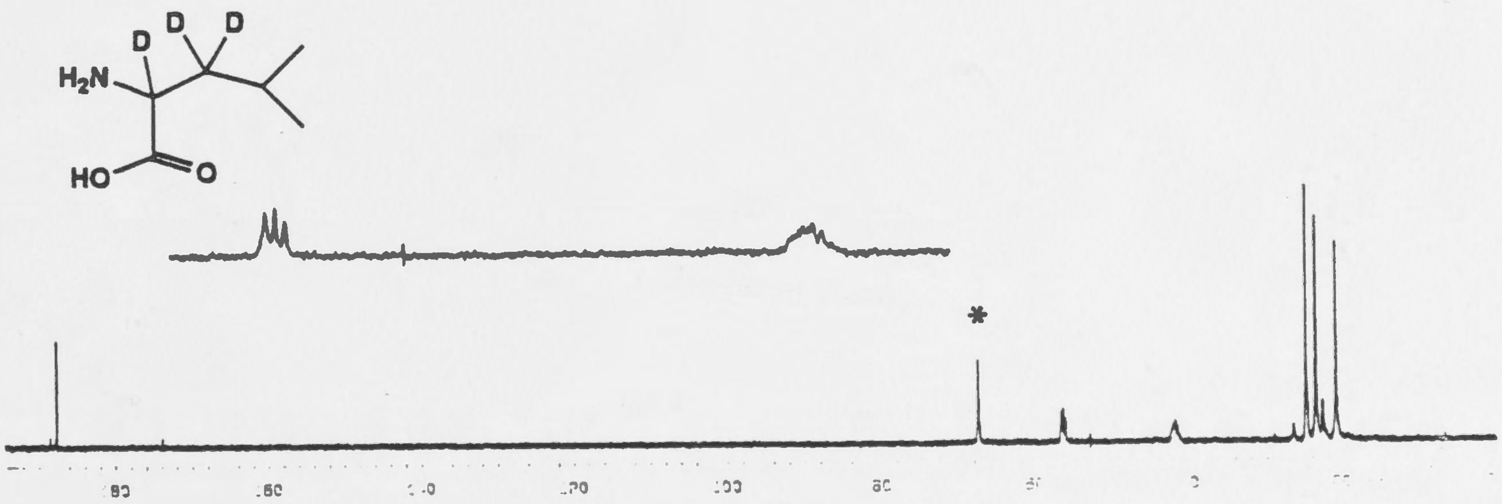


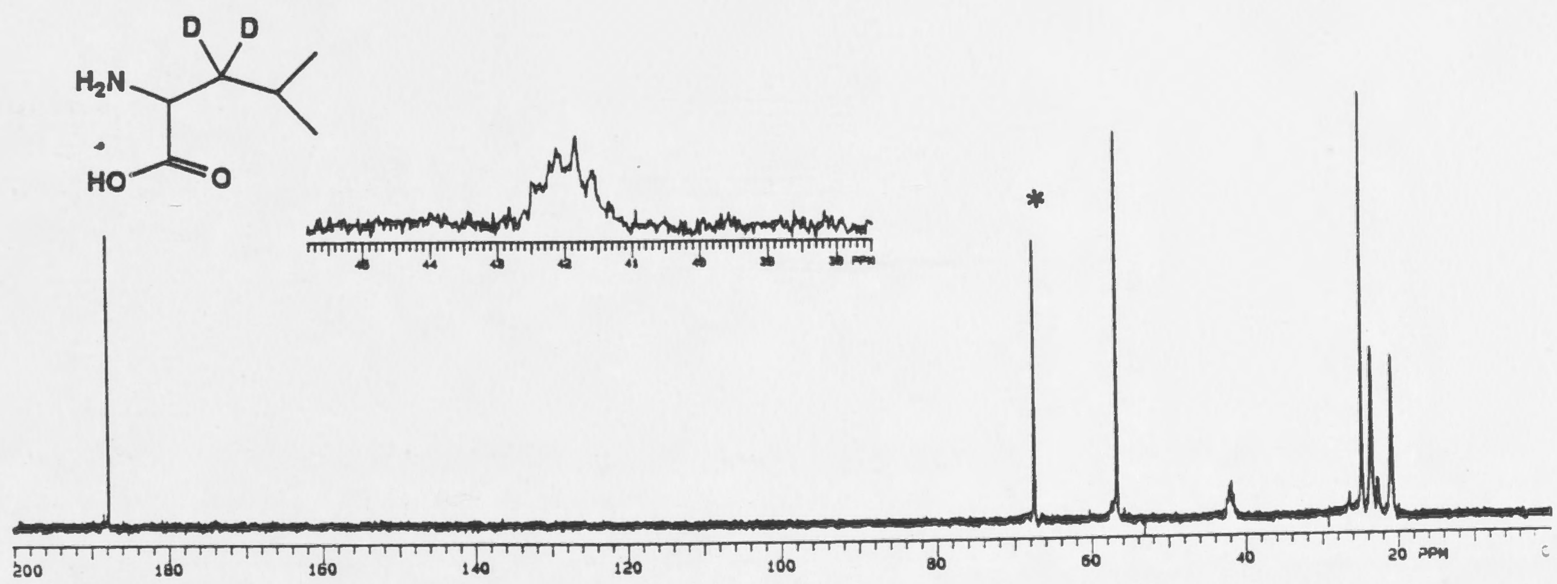
## Appendix V: Notes on the technique of electrophoresis.

Electrophoresis is a commonly used biochemical technique for separations of large biomolecules such as proteins and DNA. Separation relies on the charge and size of the molecules. Gel-electrophoresis, on agarose or acrylamide gels, was the technique used in these experiments. The size of the pores within the gel can be controlled by the quantity or nature of gelling agent used in the experiment. The smaller the pores the slower the movement of large molecules through the gel. The gel is kept moist by a buffer with a pH which causes the macromolecules being separated to have a net negative charge. An electric current (generally ~ 300 V) is passed through the gel to allow separation of the molecules on the basis of charge as well as size. Coordinated Co(III) needed to be removed in the experiments described in Chapter 6 because their positive charge would cause the metal ion-DNA complex to move in the opposite direction in the gel. The samples to be separated are placed in small, preformed wells at one end of the gel. During electrophoresis the molecules travel down the length of the gel (in 'lanes'). The bands are detected by the addition of compounds such as ethidium bromide, brightly coloured or fluorescent dyes or by incorporation of radioactivity labelled units in the macromolecules. In these experiments ethidium bromide, an aromatic compound that intercalates with DNA and which is strongly uv-active, was added to the gels. The gels were exposed and photographed in uv-light to produce the photographs in this thesis.

# Appendix V1: $^{13}C$ nmr spectra of deuteriated amino acids







Conditions for all spectra: $D_2O$, *1,4 dioxane